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## Column Chromatography of Histaminase.

By

B. SWEDIN.

Received 2 October 1957.

In recent years the metabolism of histamine has been studied with isotope techniques by SCHAYER and co-workers (1956) who made several interesting discoveries. They found for instance that histamine is eliminated from the organism by a united action of at least three different enzyme systems, one of which is the classical histaminase. For further studies of the problem it is most important to get the enzymes as pure as possible.

I should like to give a report on an improved preparation method of histaminase. The use of a chromatographic step in the method gave a good yield with a high purification.

### Method.

In the first step histaminase was prepared from pig kidneys as previously described by ARVIDSSON, PERNOW and SWEDIN (1956). This procedure involved heating of the homogenate of pig kidney cortex to + 62° C for 10 min. and precipitations with acetone and neutral saturated ammonium sulphate. The fraction obtained when the concentration of ammonium sulphate was increased from 33 to 67 per cent saturation was dialyzed against 5 per cent sodium chloride. The yield at this step of preparation was only 10 per cent.

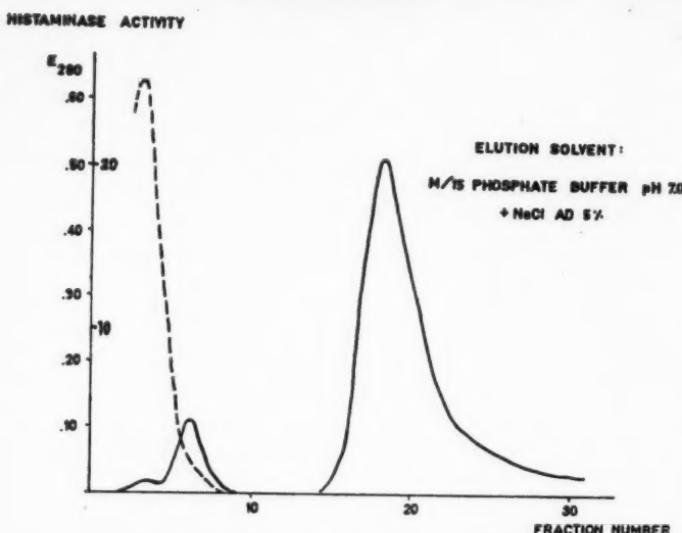
ALUMINA COLUMN

Fig. 1. Chromatography of histaminase on alumina column. Continuous curve =  $E_{280}$ . Curve of short dashes = enzyme activity.

In the next step of the preparation a chromatographic column of alumina (according to BROCKMANN) 4 cm  $\times$  20 cm supported by a sintered glass disc was used. The alumina was equilibrated with a 0.0067 M phosphate buffer solution (SØRENSEN) giving a final pH of 7.0. Material for chromatography (about 300 mg of protein) was dissolved in or dialyzed against the buffer before application to the column. The elution was carried out with a ten times more concentrated phosphate buffer of 0.067 M, which had a pH of 7.0 and contained 2.5 per cent sodium chloride. The rate of flow during the elution was 40 ml/hr. and the effluent fractions were collected in volumes of 10 ml by means of an automatic fraction collector.

The light absorption at 280 m $\mu$  was determined for each fraction in a Beckman DU spectrophotometer with the actual buffer solution as a blank.

The histaminase activity was assayed by a manometrical method previously described (SWEDIN 1943) and expressed as mg

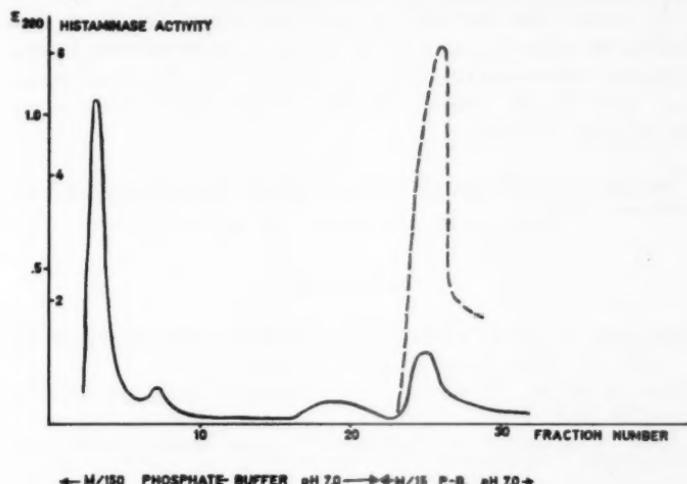
DEAE-CELLULOSE COLUMN

Fig. 2. Chromatography of histaminase on DEAE-cellulose column. Continuous curve =  $E_{280}$ . Curve of short dashes = enzyme activity.

of histamine diphosphate oxidized by one mg organic material during one hour at  $+38^\circ\text{ C}$  and pH 7.7 (mg/mg/hr.).

In this way, the results shown in Fig. 1 were obtained. It is seen that part of the material applied to the column appeared in the effluent soon after the beginning of the elution and that these fractions contained all the enzyme activity. During the continued elution a second much bigger peak appeared but without any activity. In the chromatographical step the enzyme activity of the preparation increased from 0.36 mg/mg/hr. to 25.2 mg/mg/hr. (*i. e.* 70 times). The yield at this step of preparation was 53 per cent. The enzyme preparations keep well at  $-20^\circ\text{ C}$ .

A column 1 cm  $\times$  12 cm of DEAE-cellulose was also used. The cellulose was prepared according to SOBER and PETERSON (1954) and equilibrated with a 0.0067 M phosphate buffer solution (SØRENSEN) pH 7.0. The total protein content of the material applied on the column was 20 mg. The elution was first carried out with a 0.0067 M phosphate buffer pH 7.0. After 22 fractions

had been collected, the eluent solution was changed to a 0.067 M phosphate buffer, pH 7.0. The results are reproduced in Fig. 2. It is seen that only inert protein was eluted by the weaker buffer, but after changing to the stronger buffer solution a peak appeared which contained the enzyme activity. The purification and yield on the DEAE-cellulose column was as good as on the alumina column.

This work has been supported by a grant from Stiftelsen Therese och Johan Anderssons Minne.

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## An Improved Method for Drop Recording of Arterial or Venous Blood Flow.

By

PERCY LINDGREN.

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The method for photoelectric drop recording of blood flow that has been used for several years at the Pharmacology Department was originally devised for measurement of venous outflow (CLEMENTZ and RYBERG 1949; HILTON 1952; HILTON and LYWOOD 1954; LINDGREN and UVNÄS 1954). The method, which is useful in anesthetized and heparinized animals, involves, in principle, cannulation of the vein in which the flow is to be recorded, and directing the blood by plastic tube to a photoelectric drop recorder, which in turn operates an ordinate recorder. From the drop chamber the blood returns to the cannulated proximal end of the same vein. The use of a closed system, as pointed out by LINDGREN and UVNÄS (1954), permits recording of arterial blood flow too. However, the method has several drawbacks when employed for this purpose, such as the Windkessel effect produced by the air enclosed in the drop chamber. The pulse waves have a marked influence on the actual release of drops from the nozzle, for which reason they drip at somewhat irregular intervals even when the flow is constant. The effect of this, particularly during variations in flow, is to impart a step-like appearance to the curve. Furthermore, changes of blood pressure give rise to compression or expansion of the air in the drop chamber; in some types of experiments these blood pressure variations may amount to 50—100 mm Hg. In such cases there will be a transient error in measurement amounting to several drops.

The air volume in the drop chamber used by LINDGREN and UVNÄS (1954) was approximately 3 ml; the total volume was 4—6 ml. Thus, for example, a rise of blood pressure from a basal level of 120 mm Hg to 180 mm Hg means that the air volume will be compressed from 3 ml to 2 ml; *i. e.*, about 17 drops too many will be recorded.

FOLKOW has attempted to eliminate these shortcomings by means of a micro-modification (FOLKOW, 1956). His drop chamber has a total volume of only 1.5 ml, including 1.0 ml air. This procedure reduces the above-mentioned error in measurement to only a few drops, but does not eliminate the irregularities in the intervals between drops. When such a small drop chamber is used in an experiment of long duration, moreover, blood that has splashed against the walls and dried may complicate the photoelectric recording.

For the purpose of eliminating these drawbacks with the original method, the following modifications have now been made. *Instead of using air in the drop chamber, the drops fall through an incompressible fluid.* — If, however, this fluid is to be suitable for the procedure in question, it must

1. be colorless and transparent;
2. be insoluble in blood;
3. have a specific gravity lower than that of blood;
4. have such surface tension conditions that drops form; and
5. be biologically indifferent.

Silicone DC 200/0.65 cS, specific gravity 0.75, was found to be ideal for this purpose.

This modified method has been employed, with very favorable results, for recording of both arterial and venous blood flow in rabbits, cats and dogs.

The new drop chamber is depicted in Fig. 1. It is made of transparent plastic and, aside from the substitution of silicone for air, differs in principle from earlier models only in one respect: The drop chamber and inlet and outlet tubes are enclosed in a heating shell containing circulating water, thus enabling the blood to be maintained at exactly the required temperature during its extracorporeal circulation. This is of no major importance in venous recording, but is essential in arterial recording.

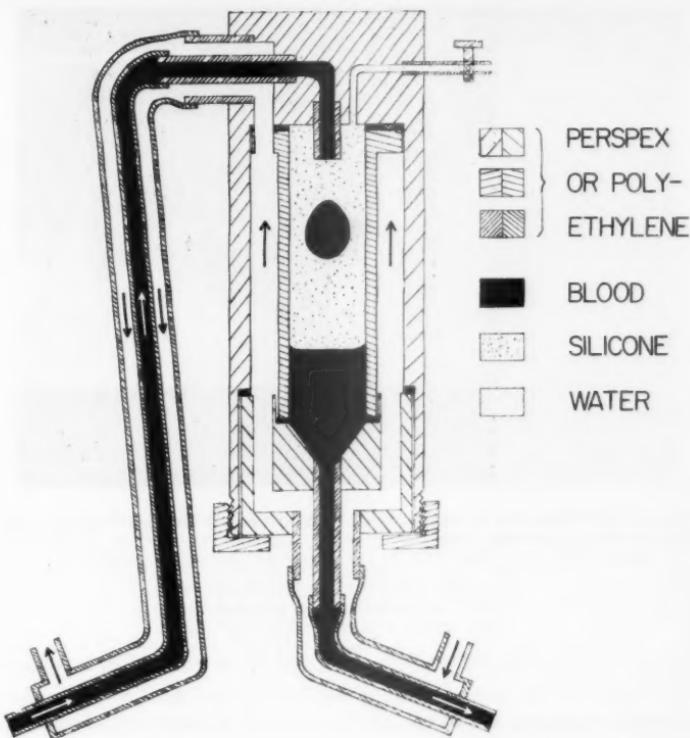


Fig. 1. Schematic drawing of the drop chamber.

A comparison of Fig. 2 and 3 illustrates some advantages of the silicone method over the air method. The blood flow in the femoral artery has been recorded in an anesthetized, heparinized cat.

In Fig. 2 a 5 ml drop chamber with 3 ml air was used. (The impulses from the photocell operate, in the conventional manner, an ordinate recorder that records the intervals between drops.) Owing to the Windkessel effect and interference between systolic pulse and rate of drip, the intervals between drops vary, the result being a curve of irregular contour that complicates quantitative reading of the blood flow. This uneven drip rate is especially marked during changes of flow. To illustrate this, intraarterial injections of acetylcholine were given — which induces pronounced

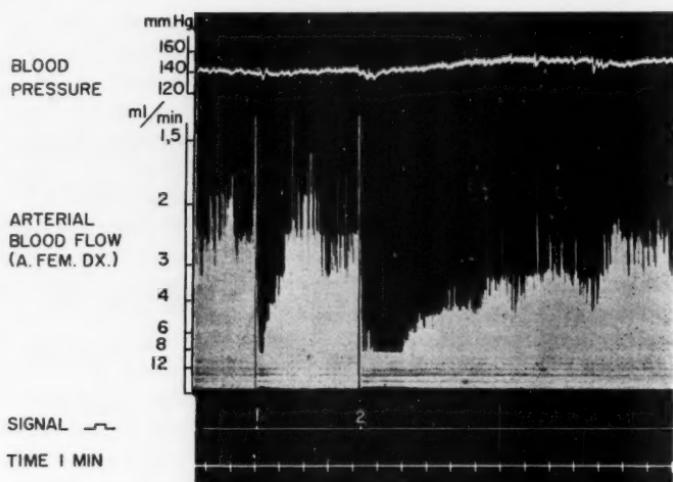


Fig. 2. Cat 2.6 kg. Dial 70 mg/kg. Muscular blood flow in the right hind leg (the leg was skinned and the paw tied off with a ligature), recorded in the femoral artery. *Air drop chamber*. Note the irregularity of the drop intervals.

1. Acetylcholine 0.1  $\mu$ g—0.1 ml injected into the femoral artery peripheral to the inflow from the recording unit.
2. Na-acetrizoate 50 %—0.1 ml, i. a.

though transient vasodilatation — and of sodium acetrizoate, a contrast medium designed for arteriography which has been found to have marked vasodilator characteristics (LINDGREN and TÖRNELL, 1957) and also has an effect of longer duration.

In Fig. 3 (same animal and drop chamber) all air has been replaced by silicone. Since no Windkessel effect is now present, the intervals between drops are quite uniform and the curve has a far more regular appearance.

Neither with arterial nor with venous recording has the silicone method shown any appreciable disadvantages by comparison with the air method. The resistance in the recording apparatus is more or less equal in the two methods. The resistance in the drop chamber (with inlet and outlet tubes) will be seen from the diagram in Fig. 4. The values, as shown, exceed by about 20 per cent those reported (LINDGREN, 1955) for an air drop chamber. However, the blood flows commonly found in small laboratory animals seldom exceed 10—15 ml per minute, and below this

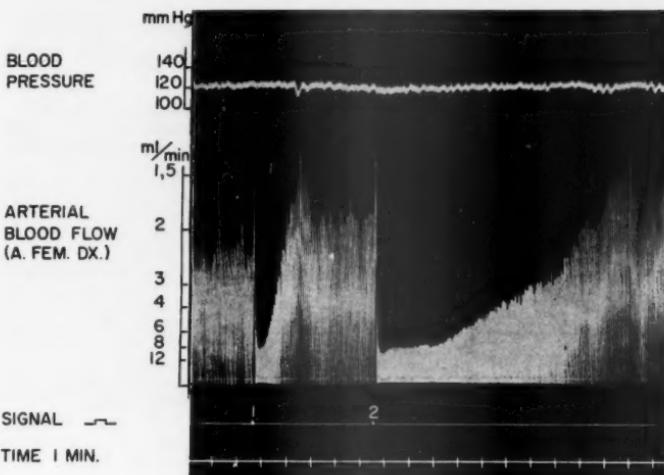


Fig. 3. Cat 2.6 kg. Dial 70 mg/kg. Muscular blood flow in the right hind leg, recorded in the femoral artery. *Silicone drop chamber.*

1. Acetylcholine 0.1  $\mu$ g—0.1 ml, i. a.
2. Na-acetrizoate 50 %—0.1 ml, i. a.

level the resistance is negligible both in arterial and in venous recording. In measurement of greater flows the resistance will be substantially reduced if tubes and cannulae of somewhat larger caliber are used.

Silicone, as mentioned above, is fully indifferent relative to blood. This has also been pointed out by DAWES, MOTT and VANE (1953), who used silicone in direct contact with blood in their density flowmeter. From a physiologic point of view, moreover, the recording apparatus does not appear to have any disturbing effect on the circulation peripheral thereto. Numerous physiologic and pharmacologic experiments have confirmed that the blood vessels in the region studied retain the same characteristics, the same susceptibility to various drugs, etc. as in venous recording. In testing of drugs by intra-arterial injection with respect to their vascular effects it seems, on the contrary, advantageous to have arterial rather than venous recording. One of the advantages is that the injected drug is distributed to the *whole* of the region in which the blood flow is measured, and *only* to that region. In venous recording, major importance often attaches to

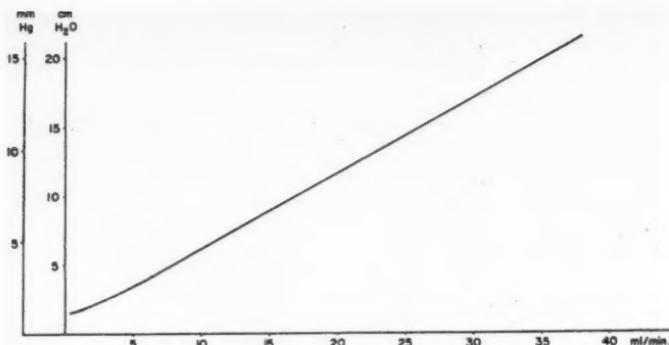


Fig. 4. Diagram showing the resistance in a drop chamber (and plastic tubes, length 30 cm, inner diameter 2.0 mm) at different rates.

anastomoses between different collecting veins; in many cases this may cause the results to vary and complicate their interpretation, especially in quantitative evaluation.

### Summary.

An improved method for drop recording of blood flow is described. Its principle is that the blood drops collect and fall through a colorless incompressible fluid (silicone) instead of through air. The principal advantages of the silicone drop chamber over the original air drop chamber are largely the following:

1. The method is equally suitable for arterial and for venous blood flow.
2. The large drops, 7—9 per ml (as compared with 17—20 per ml when the drops fall in air from the same nozzle), enable relatively large blood flows to be recorded; 30 ml per minute has been recorded without difficulty.
3. No blood can splash on the chamber walls and thus affect the light intensity; the photocell may be quite sensitive to such changes.
4. There is no Windkessel effect which is of special importance in arterial recording. Pulse waves have less influence on the release of drops from the nozzle. The intervals between drops are almost as regular as those in recording of venous blood flow.

5. There are no errors in measurement during rapid and extreme changes of blood pressure.

The method reported here was elaborated in investigations supported by a grant from the Therese and Johan Andersson Foundation, Stockholm, for which grateful acknowledgement is made.

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## Cholinesterase Content of Certain Regions of the Spinal Cord as Judged by Histochemical and Cartesian Diver Technique.

By

E. GIACOBINI and B. HOLMSTEDT.

Received 3 October 1957.

The purpose of the present investigation has been to find out if any differences in levels of cholinesterase (ChE) activity exist within a comparatively limited group of neurones in the spinal cord of the rat.

Histochemical techniques offer a possible solution to this problem. KOELLE (1954) using the thiocholine method has indicated such a difference in ChE activity in different neurones of the spinal cord of cat. The thiocholine method for histochemical determination of cholinesterase activity has recently been critically investigated by HOLMSTEDT (1957) who has emphasized the necessity for biochemical controls using solutions of the same composition as those employed histochemically. It has been our purpose to see if this modified histochemical technique could conclusively demonstrate differences in cholinesterase activity in cells of the spinal cord, as has been successfully done in sympathetic ganglia (HOLMSTEDT and SJÖQVIST 1957).

Cholinesterases of different species are known to vary in their inhibitor specificity. For this reason, *in vitro* control experiments

were carried out with rat brain homogenates and Mipafox, the inhibitor found most useful in earlier work on cat tissues. Experiments with partially purified preparations from electric organ and human serum, representing the two main cholinesterase types, were included for comparison.

In order to get a quantitative evaluation of the ChE activity in the different neurons a sensitive biochemical method for the determination of ChE activity in single nerve cells (GIACOBINI and ZAJICEK 1956) has been employed in parallel experiments with the same group of cells.

### Material and Methods.

Cervical enlargements of the spinal cord of albino rats, weighing between 150 and 200 g, were used for all histochemical and microchemical experiments. After decapitation of the animal the cord was exposed by cutting the vertebral laminae. The cervical part was then quickly dissected out and put into Ringer's solution. Homogenates of brain and spinal cord were used for the macrochemical experiments.

#### *Quantitative macrochemical method.*

The details of the macrochemical method have been described by HOLMSTEDT (1957). The technique used was the electrometric method by TAMMELIN et al. (1951, 1952, 1953). Only relative values of hydrolysis were determined. The original solutions used by KOELLE were employed with the modification that the NaH-maleate buffer had to be diluted to make electrometric determination possible.

The composition of the final reaction mixture was as follows:  $MgCl_2$ , 0.04 M; glycine, 0.0005 M;  $CuSO_4 \cdot 5 H_2O$ , 0.0011 M; NaH maleate, 0.167 M; NaOH, 0.0125 M; acetylthiocholine iodide (AcThChI), 0.004 M.

*Enzyme preparations.* Partially purified human serum and electric organ ChEs were as described by HOLMSTEDT (1957). Rat brain homogenates contained 50 % w/v tissue in 0.9 % w/v NaCl homogenized at 12,000 r. p. m. for 10 min.

*Inhibitor.* Mipafox (bismonoisopropylamidophosphoryl fluoride) was added to the enzyme exactly 30 min. before the addition

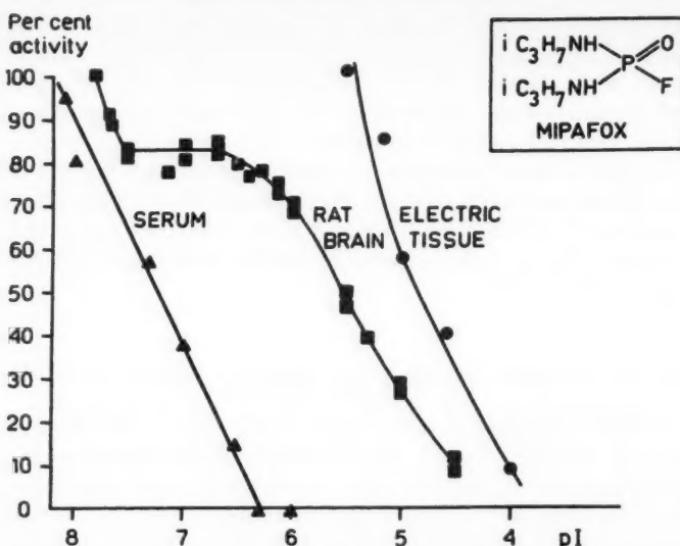


Fig. 1. Inhibition curves with Mipafox for two partially purified enzymes, electric tissue (AcChE) and Cohn's serum fraction (BuChE). Ordinate = per cent ChE activity of control. Abscissa = negative log. molar concentration of inhibitor (pI). The inhibition curve for rat brain homogenate fits between those of AcChE and BuChE. Substrate: Acetylthiocholine iodide 0.004 M.

of the substrate. The final volume of the reaction mixture was 5 ml.

#### Quantitative microchemical method.

An isolated segment ( $C_5$ ,  $C_6$  or  $C_7$ ) about  $500 \mu$  thick, was cut out and placed in the microdissection chamber. Single anterior horn cells were isolated from an antero-lateral group (A in Fig. 2) according to the technique of micromanipulation recently described (GIACOBINI 1956). This group was selected because of its relative accessibility. In order to avoid contamination with the cytoplasmic material present in the preparation a single cell was transferred by means of a micropipette to a drop of paraffin oil lying on a microscope slide. The isolated cell preparation was then inspected under high magnification in phase contrast and injured cells discarded. The maximum ( $m_1$ ) and minimum ( $m_2$ ) diameters of the cell body were recorded by means of a micrometer

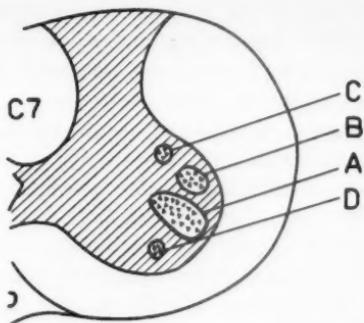
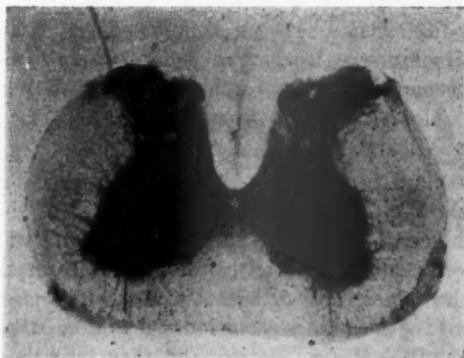


Fig. 2. a. Schematic representation of the four principal groups of large anterior horn cells (C<sub>7</sub> level) innervating the fore limb muscles of the rat. The cell column A innervates predominantly extensor muscles. The cell columns B and C are correlated with adduction and D with flexion.



b. Histochemical survey of the spinal cord at the same level. Heavily stained regions localized in the anterior and lateral horns. Magnification 20 times.

eyepiece (Baker) at 900 x. The cell volume and surface were calculated on the assumption that the cell was a sphere with a diameter given by the expression  $\sqrt{m_1 m_2}$ . This diameter is referred to as the 'computed diameter'. The application of this formula produces a mean error of about 25 % as compared with more adequate methods of calculation (MICKLEWRIGHT, KURNICK and HODES 1953); thus the values reported have only a relative significance.

The cell which was to be analyzed was sucked into a diver having a gas volume of about  $0.2 \mu\text{l}$  and weighing about 1 mg. In some experiments smaller divers were employed weighing 10—100  $\mu\text{g}$  and having gas volumes of 0.01—0.08  $\mu\text{l}$ .

The quantitative determination of acetylcholinesterase (AcChE) (specific, true cholinesterase) and non-specific or pseudocholinesterase was performed according to the procedure previously described (GIACOBINI and ZAJICEK 1956; GIACOBINI 1957). The thiocholine formed as a result of the hydrolysis of AcThCh has acidic properties (HANSEN 1956; AUGUSTINSSON and ISACHSEN 1957) therefore the amount of  $\text{CO}_2$  evolved may not exactly correspond to the absolute amount of ester split. For a detailed discussion of this see GIACOBINI (1958). Since DAVISON (1953) has shown that rat brain non-specific ChE is a propionoChE, *i. e.*, has an acyl-group maximum at propionate, this enzyme will be referred to as propionocholinesterase (PrChE). The sensitivity of the technique employed was  $10^{-6} \mu\text{l}$   $\text{CO}_2$  with 10% accuracy. In order to differentiate between AcChE and PrChE the selective inhibitor Mipafox was used at a concentration of  $5 \times 10^{-6} \text{ M}$  which, as demonstrated by biochemical controls performed on brain and spinal cord ChE from rat (see Fig. 1) completely removes PrChE activity.

#### *Histochemical method.*

The technique described by HOLMSTEDT (1957) was followed. Fresh frozen sections were cut transversally from the cervical region of the spinal cord with a thickness of 10 microns. After pretreatment in a storage solution they were transferred to Mipafox  $4 \times 10^{-6} \text{ M}$  for 30 min. and then into the incubation solution, for periods from 5 min. up to 3 hours. In some experiments the sodium sulphate was replaced by 0.9 % w/v NaCl. The sections were not treated with ammonium sulphide but after incubation transferred to 10 % formalin for 10 min. and then, after a faint counter-staining with hematoxylin-eosin, dried and mounted in balsam. Photomicrographs were taken of sections incubated for various time intervals and are reproduced untouched.

#### **Results.**

Both AcChE and PrChE are present in the central nervous system and other tissues of the rat. This has been proved both

with the use of selective substrates and selective inhibitors (ORD and THOMPSON 1951; ALDRIDGE 1953-54; DAVISON 1953). Inhibition curves with Mipafox where the enzyme is rat brain and the substrate acetylthiocholine have not previously been performed and therefore this inhibition curve has been run in the histochemical solution.

### 1. Inhibition curve with Mipafox (Fig. 1).

The inhibition curves for two partially purified enzymes Cohn's serum fraction R. d. c 2: IV-6, non specific ChE or butyrocholinesterase (BuChE) and electric tissue AcChE, have been plotted in Fig. 1. Acetylthiocholine was the substrate in all cases. From previous investigations (ALDRIDGE 1953-54; DAVISON 1953; HOLMSTEDT 1957) it is known that Mipafox is a selective inhibitor of non specific ChE. The inhibition curves for the two enzymes are well separated and a range of inhibitor concentrations exist at which one type of enzyme is completely inhibited and the other unaffected.

When rat brain homogenate is used as source of enzyme under identical conditions the curve lies between those of AcChE and BuChE. A plateau is visible indicating where one type of enzyme is completely inhibited and the other unaffected. This curve confirms the results of previous investigations (ALDRIDGE 1953-54; DAVISON 1953) that rat brain contains approximately 20 per cent PrChE and 80 per cent AcChE. The curve for rat brain cholinesterase does not coincide with those of the purified enzymes and the concentration range corresponding to 100 % inhibition of PrChE and zero inhibition of AcChE falls below that observed with the purified enzymes. At Mipafox concentrations higher than  $10^{-6}$  M the enzyme activity decreases parallel with the curve for electric tissues. The inhibitor concentration used histochemically ( $4 \times 10^{-6}$  M) thus assures a complete inhibition of PrChE. Only AcChE was studied in the present investigation.

### 2. Histochemical results.

The motor cell grouping has been established in the present work from a series of sections of the cervical spinal cord and the results have been compared with a previous histological investigation on the same material (GOERING, 1928). Confirming the results of GOERING (1928) in the seventh segment, four principal groups could be distinguished which for simplicity have been indicated

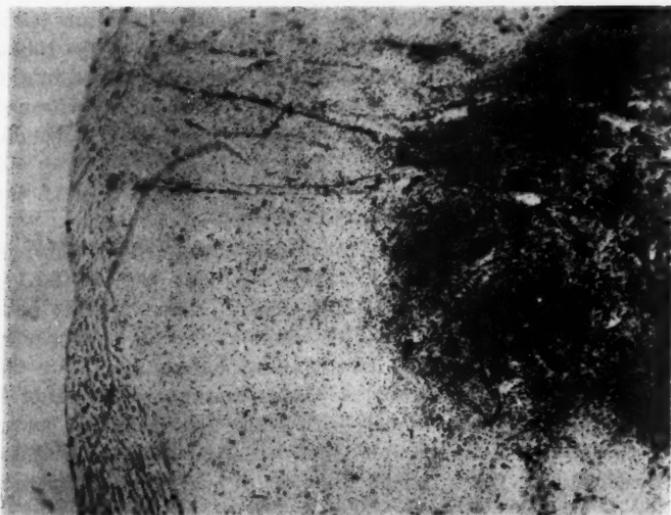


Fig. 3. Antero-lateral part of the anterior horn (cervical tract of the spinal cord of rat). Staining, modified thiocoline method. The copper thiocoline crystals are visible in the motoneurones and can be traced along the neurites issuing from these cells. Magnification 120 times. Incubation time, 60 min.

by the letters A, B, C, D. Fig. 2 a shows a schematic representation of the columns of cells at C 7 which innervate the fore limb muscles of the rat. Group A (antero-lateral) contains the largest number of cells, has an oval contour with its long axis disposed in the frontal plane and is uniform in magnitude in the sixth and seventh cervical segment but enlarged in the eighth cervical and first thoracic segment. This group has been shown to be related to extension (GOERING 1928). The cells of this group are more accessible to dissection, and have therefore been chosen as material for the present investigation. A histochemical survey of the spinal cord (Fig. 2 b, Section between C 6—C 8) shows heavily stained regions strictly localized to the anterior and lateral horns. The above-mentioned cell groups are also visible on the section. The neurites cut longitudinally on the section show a histochemical reaction and are clearly visible. As seen from the picture no cells in the posterior horns are stained histochemically. Since the spinal cord has been faintly counterstained with hematoxylin-eosin, the grey matter including the posterior horn stands out

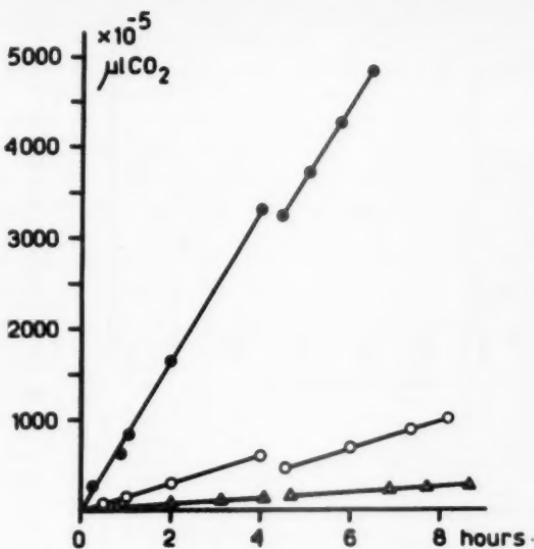


Fig. 4. Activity curves of three anterior horn cells having the following computed diameters:  $30 \mu$  (empty circles),  $24.4 \mu$  (filled circles),  $18.6 \mu$  (triangles). The intervals in the curves indicate where the experiments have been interrupted for the addition of the inhibitor (Mipafox  $5 \times 10^{-6} \text{ M}$ ).

from the rest of the cord. There is a distinct difference between the cell bodies stained histochemically which are found only in the anterior and lateral horns and the nuclei stained by the hematoxylin in the posterior horn.

Fig. 3 shows a magnification of a cell group in the anterior horn with the same localization in the spinal cord as in the previous picture. In this picture, the part from which cells have been taken for the Cartesian diver experiments, the antero-lateral part, can be studied in detail. In spite of a certain diffusion of the histochemical end product the cholinesterase activity in the motor cells is clearly visible and the copper thiocholine sulphate crystals can be traced along the neurites issuing from these cells. This and other unpublished histochemical sections show in agreement with KOELLE (1954) different enzyme activities in various cells. However, these differences are small, and in view of variations in the sharpness of focus in different parts of the field a quantitative judgement hardly seems justified.

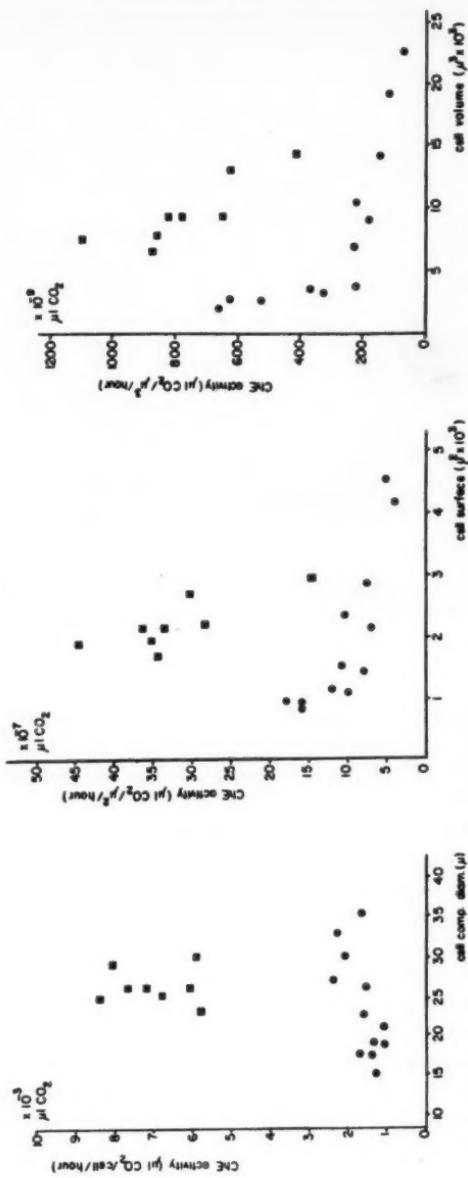


Fig. 5. ChE activity in  $\mu\text{l CO}_2/\text{hour per cell}$  (A), unit of surface (B) and volume (C), and volume (D). (○) cells belonging to the first group, (■) cells belonging to the second group (see Table 1 and text).

Table 1.

*Cholinesterase activity of single intact anterior horn cells isolated from the antero-lateral group of the spinal cord of rat.*

Exp. no.	Computed diam. in $\mu$ ( $\sqrt{d_1 \times d_2}$ )	Cell surface in $\mu^2$ ( $\times 10^{-9}$ )	Cell volume in $\mu^3$ ( $\times 10^{-3}$ )	Activity $\mu\text{l CO}_2/\mu^2/\text{h.}$ ( $\times 10^{-7}$ )	Activity $\mu\text{l CO}_2/\mu^3/\text{h.}$ ( $\times 10^{-9}$ )	Total activity $\mu\text{l CO}_2/\text{cell/h.}$ ( $\times 10^{-3}$ )
19	35.6	4.140	24.10	4.32	69.60	<sup>1</sup> 1.68
23	25.9	2.100	9.12	7.44	171.2	1.56
29	27.2	2.310	10.50	10.44	228.8	2.40
44	30.0	2.800	14.10	7.48	148.8	2.10
49	33.2	3.450	19.10	6.76	121.6	2.33
51	21.2	1.410	5.00	7.80	210.0	1.10
52	17.2	0.933	2.62	15.72	524.0	1.40
54	17.3	0.957	2.72	18.12	624.0	1.70
55	18.6	1.080	3.35	10.20	328.0	1.10
59	19.1	1.140	3.68	11.92	388.0	1.35
62	15.6	0.813	1.99	16.12	657.6	1.31
63	22.0	1.510	5.55	10.72	292.8	<sup>1</sup> 1.62
				$M_2 = 10.45 \pm 1.25$ $\delta = 4.22$	$M_2 = 308 \pm 57$ $\delta = 192.7$	$M_2 = 1.63 \pm 0.129$ $\delta = 0.425$
30	26.1	2.210	9.86	33.80	778.4	<sup>1</sup> 7.20
34	23.2	1.680	6.56	34.40	880.0	<sup>1</sup> 5.80
36	24.9	1.940	7.93	35.16	856.0	<sup>1</sup> 6.80
38	29.2	2.650	13.1	30.53	625.6	8.11
40	24.4	1.860	7.63	44.80	1096	8.40
41	30.2	2.900	14.4	14.84	412.0	5.94
42	26.2	2.150	9.45	28.40	648.0	6.10
46	26.1	2.120	9.35	36.40	824.0	7.70
				$M_1 = 32.25 \pm 3.08$ $\delta = 8.54$	$M_1 = 765 \pm 72.5$ $\delta = 205$	$M_1 = 7.00 \pm 0.32$ $\delta = 0.915$

<sup>1</sup> AcChE activity alone. Statistical results were computed from the following formulae: the standard deviation,  $\delta = \sqrt{\frac{\sum a^2}{n-1}}$  where  $a$  is the deviation from the mean value and  $n$  the number of observations; The mean errors of the arithmetical means  $M_1, M_2 = \pm \frac{\delta}{\sqrt{n}}$ .

No exact intracellular localization of the esterase activity has been attempted. A detailed study of the topographical distribution of the enzyme has shown (GIACOBINI 1957) that AcChE is localized predominantly in the cytoplasm and in the axon but partly also in the nucleus.

### 3. Results with Cartesian diver technique.

Table I shows the results of experiments on cells belonging to cell group A in Fig. 2. The ChE activity of single intact anterior horn cells is presented together with their computed diameters. The relationship of the enzyme activity to cell surface and volume is also given. All the cells investigated show a measurable cholinesterase activity in contrast to the cells of sympathetic and spinal ganglia in cats and rats (GIACOBINI 1957; HOLMSTEDT and SJÖQVIST 1957).

The Cartesian diver technique also shows that the ChE activity of the single cell is many times higher in the anterior horn than in the ganglion cells. As seen from Table I and Fig. 5 no direct correlation could be demonstrated between ChE activity and either cell diameter, cell surface or cell volume. The Tables display two significantly different groups of cells with respectively high and low ChE activity. If the computed diameter is taken as a reference one group of cells has approximately four times the activity of the other.

The high activity of cells pretreated with the selective inhibitor Mipafox shows that the main contribution to the total ChE comes from AcChE. The pretreated cells have been marked with 1 in the table. Fig. 4 demonstrates the total AcThCh splitting activity per unit time of three cells chosen at random. The interruptions of the curves indicate where Mipafox  $5 \times 10^{-6}$  has been added. The technical procedure is described by GIACOBINI (1958). This inhibitor concentration gives a complete inhibition of non specific ChE under the experimental conditions described (GIACOBINI and ZAJICEK 1956). As is evident from the identity in slope of the curves before and after the break the splitting of acetylthiocholine continues at the same rate after addition of inhibitor. Two of the cells in Fig. 4 (filled circles and empty circles) have approximately the same dimensions but differ significantly in enzyme activity. The third cell (triangles) has one of the smallest computed diameters investigated and also displays a low enzyme activity.

### Discussion.

#### *Comparison of diver experiments and histochemical technique.*

Although the thiocholine method has been modified to give less diffusion and better localization (for details see HOLMSTEDT 1957)

some factors still make a quantitative estimation of the enzyme activity hazardous. These may be summarized as follows:

- a) the opacity of the metal salt (copper thiocholine sulphate) precipitated at the site of enzyme activity,
- b) the difficulty in obtaining an equal sharpness of focus in different parts of the section,
- c) diffusions of the end-product which cannot be entirely eliminated.

Sodium sulphate, originally introduced by KOELLE (1951) to minimize diffusion of the proteins was retained in some experiments; in others it was replaced by physiological saline. In agreement with what has been found biochemically by KOELLE the reaction was considerably slowed down by  $\text{Na}_2\text{SO}_4$ , also the crystals precipitated were of smaller dimensions. When  $\text{Na}_2\text{SO}_4$  was used, the copper thiocholine sulphate showed a tendency to precipitate in the neighbourhood of the cell membrane, whereas with  $\text{NaCl}$  it was more uniformly distributed over the cell surface; in the latter case the neurites were also intensely stained. The reason for these differences in localization is at present obscure, and it is doubtful which one presents the truer picture.

In sympathetic ganglia (GIACOBINI 1957) the cells display a much wider variation in ChE activity (between  $0.1 \times 10^{-8}$  and  $3 \times 10^{-8} \mu\text{l CO}_2/\text{hr.}$ ) than in the anterior horn cell ( $1 \times 10^{-8}$  to  $8 \times 10^{-8} \mu\text{l CO}_2/\text{hr.}$ ) though the absolute activity is lower in the former. It is conceivable that the wider variation in ChE activity in the cells of the sympathetic ganglia permits better histochemical differentiation in tissue sections. Another factor is also important in this connection: the high absolute values of the ChE activity of the anterior horn cells favour the rapid precipitation of large amounts of copper thiocholine sulphate making a quantitative differentiation less easy than in the sympathetic ganglia where the reaction occurs more slowly. The decrease of the incubation time of the spinal cord sections down to 5 minutes did not however significantly modify the results.

It is thus clear that quantitative assessments of cholinesterase activity can only be made from histochemical studies with great caution. That such assessments are possible is demonstrated by sympathetic ganglia where at least three types of cells can be distinguished histochemically (GIACOBINI 1956; HOLMSTEDT and SJÖQVIST 1957).

*Possible significance of cells with varying levels of enzyme activity.*

In the present investigation the Cartesian diver technique has been utilized to measure the AcChE activity of cells in a strictly localized region of the anterior horn. It appears that regardless of the computed diameter or the surface or volume of the cells they can be divided in two significantly different groups by means of their cholinesterase activity. The mean of the total ChE activity per cell of these two groups were  $1.63 \pm 0.129 \times 10^{-3} \mu\text{l CO}_2$  per hour and  $7.00 \pm 0.324 \times 10^{-3} \mu\text{l CO}_2$  per hour, or approximately a fourfold difference. This relates entirely to AcChE as demonstrated by experiments with the selective inhibitor Mipafox, Table 1.

Table 1 demonstrates that the ChE activity in relation to the cell volume varies up to more than one hundred times. In contrast to sympathetic and spinal ganglion cells all anterior horn cells show some ChE activity. This was found to be about 10—12 times higher in the anterior horn cells than in the sympathetic and spinal ganglion cells (GIACOBINI 1956—57). The curves presented in Fig. 5 call to mind the distribution of ChE activity in the cervical spinal ganglion cells (GIACOBINI 1957).

When discussing the significance of different levels of enzyme activity in the different neurones it is interesting to note that BRATTGÅRD and HYDÉN (1952) and GOMIRATO (1953) using X-ray microradiography, found a wide variation of the mass and protein content of cells belonging to the same nervous structure (Deiter's nucleus, anterior horn cells, spinal ganglion cells, Purkinje cells). It is an open question whether the "light" and "heavy" cells demonstrated by means of X-ray microradiography can in any way be correlated with the cells with "low" and "high" cholinesterase content demonstrated in the present investigation.

From accurate studies of the organization of the motor cells through a large series of vertebrates (ROMANES 1953) it would seem that the division of the cell groups is related to joint moved rather than to either the topographical position or to the specific function of the muscles innervated. A gross division of the motor cells into groups innervating flexors and extensors is, however, probably present in all mammals, even if this division is not always clear-cut. One possible though perhaps unlikely explanation of the difference in the ChE activity of the two groups of cells thus would be that they represent flexor and extensor neurones. Pharmacological evidence for a difference in sensitivity

to ChE inhibitors between flexor- and extensor neurones has been presented by several authors (for references see HOLMSTEDT and SKOGLUND 1953).

The dimensions and the specific localization of the neurones investigated in this work indicate that they are motor neurones. It is believed that motor cells of somewhat smaller size in the anterior horn give rise to the gamma efferents of the anterior roots supplying the contractile poles of the muscle spindles. Most authors (HUNT and KUFFLER 1951; ELDRED, GRANIT, MERTON and HOLMGREN 1953-55) accept the connection of the gamma efferents with "small motor cells". The question arises, if the cell bodies of smaller dimensions found in the present investigation are those from which the gamma efferents issue. Another possibility to be taken into consideration is that the smaller cells investigated are Renshaw cells.

Finally the hypothesis should be considered that the two groups of cells described correspond to the fibers innervating "slow" (red) and "fast" (white) muscle fibers. GRANIT, HENATSCH and STEG (1956) ECCLES, ECCLES and LUNDBERG (1957) and GRANIT, PHILIPS, SKOGLUND and STEG (1957) have differentiated in the cat two types of ventral horn cells the "tonic" and the "phasic" ones. It is not unlikely that the two groups of motor neurones with differences in AcChE activity can be correlated to the "tonic" and "phasic" anterior horn cells.

Regardless of what the explanation of the differences in AcChE activity may be the knowledge that within an anatomically well defined group of neurones of the central nervous system cells coexist which have different enzymic levels and which possibly react differently pharmacologically for this reason, has to be taken into consideration when the electrical properties of these neurones are investigated.

### Summary.

1. The cholinesterase content of neurones belonging to the antero-lateral group of the anterior horn of the spinal cord of rat has been investigated histochemically by means of a modified thiocholine method and microchemically by means of a Cartesian diver technique.

Biochemical controls with the inhibitor (Mipafox) run in solutions similar to those used histochemically have been made.

2. When rat brain homogenate was used as source of enzyme, with Mipafox ( $4 \times 10^{-6}$  M) as inhibitor, and acetylthiocholine as substrate (0.004 M), the inhibition curve fits between those of AcChE (electric tissue) and BuChE (Cohn's serum fraction).

3. A histochemical survey of a section of the cervical cord shows that only anterior and lateral horns are stained. The large motor neurones are heavily stained and the product of the histochemical reaction is clearly visible along the neurites. The difference in staining intensity among various cells is not sufficiently marked to allow a quantitative appreciation of cholinesterase activity.

4. By measuring the cholinesterase activity of single neurones by means of the Cartesian diver technique two significantly different groups of cells can be demonstrated. In one group the total enzyme activity per cell is approximately 4 times higher than in the other. All the cells investigated have a measurable cholinesterase activity. Experiments with a selective inhibitor demonstrated that the enzyme present in these cells is acetylcholinesterase.

5. The results are discussed in the light of what is known about the morphology and physiology of the motor cell groups of the spinal cord.

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## Unsaturated Fatty Acid Composition of Subcutaneous Fat and Liver Fat in Rats in Relation to Dietary Fat.

By

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In earlier work (CHRISTENSEN, DAM and ENGEL, 1957) we have excised and analyzed samples of subcutaneous fat tissue once a week from the same rat and followed the change with time in the composition of the depot fat under two different dietary regimens, viz. fat deficiency and supply of hydrogenated peanut oil.

In the present experiment, the same technique was applied to rats receiving various amounts of unhydrogenated peanut oil.

### Experimental.

Thirty female rats, 3 weeks old, were divided into 5 equal groups. The basal fat-free diet given was the same as that described earlier (CHRISTENSEN, DAM and ENGEL, 1957). One group received the fat-free diet without supplementation. The others were given supplements corresponding to 38, 89, 178, and 444 mg<sup>1</sup>, respectively, of peanut oil per rat per day by means of an automatic syringe. The peanut oil contained 21.8 % linoleic acid and 0.4 % linolenic acid as determined by the alkali-isomerization procedure of HAMMOND and LUNDBERG (1953).

Samples of depot fat were taken out 1, 14, 34, 56, 84, 112, 147, and 183 days after the beginning of the experiment and analyzed for polyenoic acids by the aforementioned procedure. After 183

<sup>1</sup> 38 mg peanut oil/rat/day were given in the form of three weekly doses of 88.8 mg each. The other supplements were given daily.

days of experiment the rats were killed together with 5 parallel groups of female rats which had been fed exactly as above, but from which no samples of depot fat had been taken during the feeding period. At autopsy, the livers and subcutaneous fat tissue from both sides of the inguinal region were taken out from all the rats.

The depot fat was treated as described earlier (CHRISTENSEN, DAM and ENGEL, 1957).

The liver fatty acids were isolated from the liver tissue by the following procedure:

Each liver was dissolved in a mixture of 50 ml 30 % aqueous KOH and 20 ml 96 % ethanol and heated on steam bath for 2 hours. The tissue was dissolved during the first 15 minutes. After cooling the unsaponifiable matter was extracted with light petroleum. The aqueous phase was acidified and extracted again with light petroleum. The acid extract was washed with water, dried, and evaporated to dryness in a stream of nitrogen with gentle heating. The residue was weighed and an aliquot of it was subjected to alkali-isomerization analysis.

In calculating the results for livers we have not corrected for the light absorption at 233  $\mu$  of the *non-isomerized* fatty acids, because the isolation of the fatty acids gives rise to a certain degree of absorbance at this wavelength, which would lead to an exaggerated figure for preformed conjugated fatty acids and a lowered value of non-conjugated dienoic acid.

The dienoic acid content of the subcutaneous fat — which was not isolated by saponification — was calculated in the usual manner after correcting for the "biological" preconjugation.

According to NUNN and SMEDLEY-MACLEAN (1938), the trienoic acid which accumulated during fat deficiency is different from linolenic acid but identical with dihydroarachidonic acid and with 5, 8, 11 eicosatrienoic (MEAD and SLATON, 1956). Since — according to MEAD and SLATON — the absorption of the alkali isomerized 5, 8, 11 eicosatrienoic acid is comparable both qualitatively and quantitatively to that obtained with linolenic acid, we have used the set of formulae given by HAMMOND and LUNDBERG in calculating the results of the liver fat analyses.

### Results and Discussion.

Fig. 1 shows the decline of dienoic acid in the fat tissue during the experimental period.

In all cases the percentage of linoleic acid in the subcutaneous fat declined to about 50 % of the original value during the first 13 days. During the further course of the experiment the amount of dienoic acid in the fat tissue attains a level determined by the amount of peanut oil given.

The decline with time of the concentration of dieonic acid in the fat tissue may be due to an increase of the absolute amount of fat in the adipose tissue and/or to removal of dienoic fatty acid from this tissue. Further experiments are required to decide which of these causes is the most important.

If the end points of the curves in fig. 1 are plotted against the daily amount of peanut oil given to each rat a nearly straight line

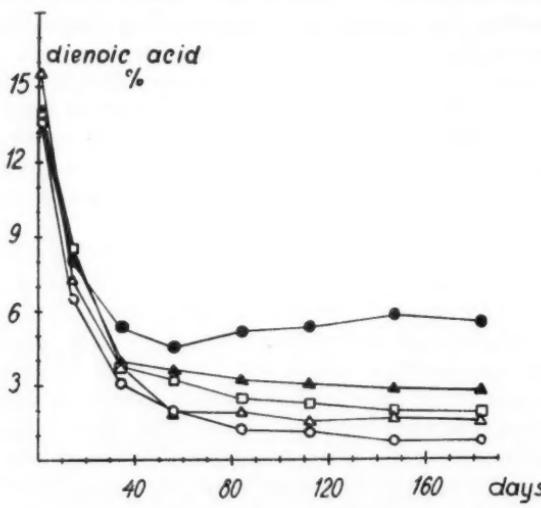


Fig. 1. Percentage of dienoic fatty acid in subcutaneous fat as a function of time from beginning of the experiment. Each point represents the average of results from 3 rats.

- no fat supplement
- △—△—△ 38 mg peanut oil per rat per day
- 89 " " " " " " "
- ▲—▲—▲ 178 " " " " " " "
- 444 " " " " " " "

is obtained. This is shown in fig. 2 (○—○—○). The corresponding points from the control groups ( $\Delta$ — $\Delta$ — $\Delta$ ) have also been plotted; it is seen that the removal of samples from the fat depots has not influenced the percentage of dienoic acid in the depot fat.

Fig. 2. also shows the percentage of tetraenoic acid at the end of the feeding period (183 days) as a function of the dose of peanut oil; like the percentage of dienoic, it follows a straight line. The slope of the curve is small.

A decrease in the percentage of trienoic acid normally seen in *organ fat* of animals fed increasing amounts of peanut oil was not seen in the depot fat. The percentage of trienoic in this fat remained almost constant, viz. about 0.3 %. The cause of this may be that a possible decline of this small value is compensated by the small amount (0.4 %) of linolenic acid in the peanut oil, or that the depot fat behaves differently from *organ fat*, such as it has been found for

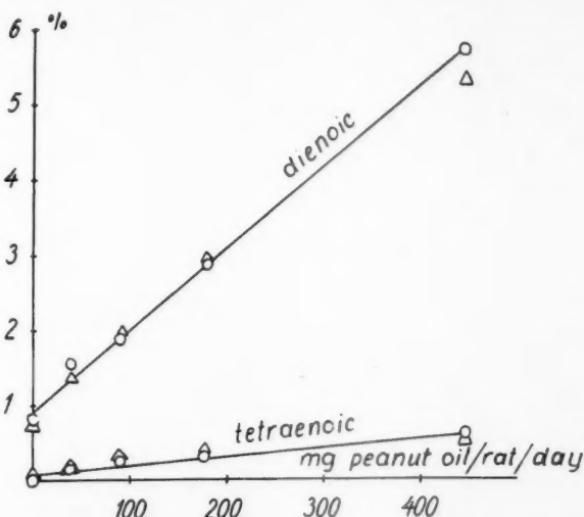


Fig. 2. Percentage of dienoic and tetraenoic fatty acids at the end of experiment (183 days) as a function of the dose of peanut oil.

○—○—○ refers to rats from which samples of fat tissue had been taken out at the intervals indicated in the text.

Δ—Δ—Δ refers to rats from which samples of fat tissue were taken out at the end of the experimental period only.

chicks in a 4-weeks feeding experiment (DAM, KRISTENSEN, KOFOED NIELSEN, PRANGE and SØNDERGAARD, 1956).

The average percentage of dienoic acid in the depot fat at the beginning of this experiment was about 14 %. Even with a daily supply of 444 mg of peanut oil, corresponding to about 88 mg of linoleic acid per rat, a decrease to about 6 % occurred. If it is permissible to extrapolate from fig. 2, the amount of peanut oil required to keep the percentage of subcutaneous linoleic acid at the initial level during 26 weeks would be expected to be about 1000 mg/rat/day, corresponding to 200 mg linoleic acid.

Fig. 3 shows the development of scaly tail, assessed as degrees from 0 to 4, corresponding to absence of symptom, mild, moderate, marked, and very marked scaliness. Each point represents an average from 12 animals. At the beginning of the experiment all the rats developed scaly tail. In the cases where peanut oil was given the scaliness declined after 50 to 90 days and disappeared after 140 days, except for a low degree in the group receiving only

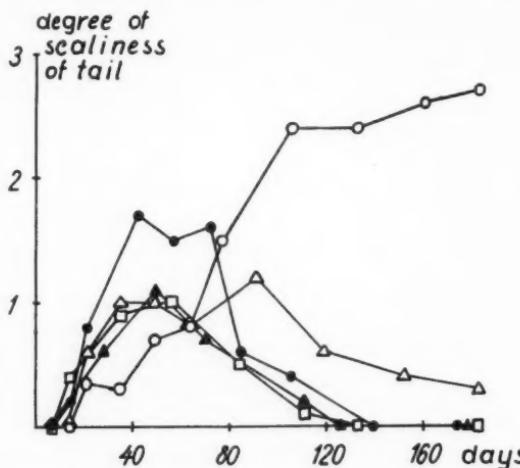


Fig. 3. Degree of scaliness of tail as a function of time after beginning of the experiment. Each point represents the average of results from 12 rats.

- no fat supplement
- △—△—△ 38 mg peanut oil per rat per day
- 89 " " " " " "
- ▲—▲—▲ 178 " " " " " "
- 444 " " " " " "

38 mg peanut oil per day. When no peanut oil was given, the scaliness developed more slowly but attained high degrees and did not decline later on.

Eighty-nine mg peanut oil/rat/day were sufficient to eliminate the scaliness completely after 133 days of experiment.

The fact that scaliness developed in the beginning of the feeding period for all doses of peanut oil used in the experiment might indicate that the requirement of linoleic acid is greater the first 10 weeks than during the following weeks. It would be interesting to see whether the amount of 1000 mg peanut oil/rat/day calculated from the curve in fig. 2 to prevent the decrease in subcutaneous dienoic acid would prevent the initial development of scaly tail.

*Scaliness of feet and scaliness of fur* were seen only in the rats reared on the fat-free diet. The development of these symptoms began after 35 days of experiment.

Fig. 4 shows the content of tri-, tetra- and pentaenoic fatty acids in liver fat from the different groups of rats at the end of the feeding period (183 days) as a function of the dose of peanut oil.

The accumulation of trienoic acid found on the fat-free diet confirms earlier findings of NUNN and SMEDLEY-MACLEAN (1938). Fig. 4 shows that with increasing dose of peanut oil the tetraenoic

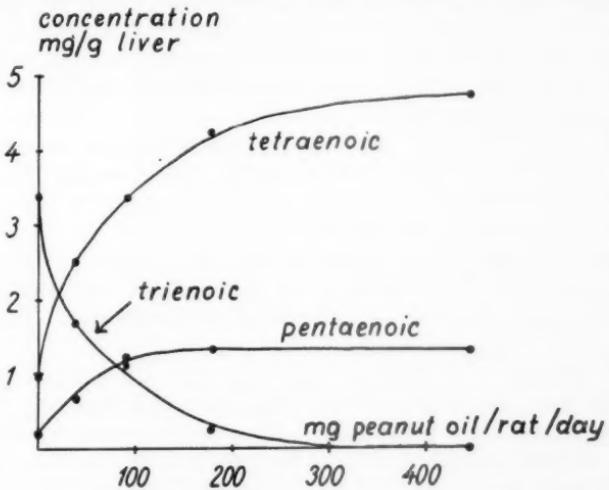


Fig. 4. Content, expressed as mg per g liver, of tri-, tetra-, and pentaenoic fatty acids as a function of the dose of peanut oil. Each point represents the average of results from 12 rats.

acid content calculated as mg/g of fresh liver increases and levels off at a maximum value (4.7 mg/g liver). Trienoic acid decreases with increasing dose of peanut oil from a level of 3.4 mg per g of liver to zero. It seems that 440 mg peanut oil per day are sufficient to secure maximum value for liver tetraenoic acid and disappearance of liver trienoic acid.

Further, from fig. 4 it can be inferred that the sum of tetraenoic and trienoic acids remains almost constant, independent of the doses of peanut oil.

This finding would be in agreement with the view held by MEAD and SLATON (1956), viz. that during fat deficiency the liver cells are able to synthesize trienoic acid from tetraenoic acid.

In a previous communication (DAM, ENGEL and KOFOED NIELSEN, 1956) it was shown that the increase in trienoic fatty acids in rats and chicks reared on fat-free diets may have originated from dienoic acid, because the decrease in dienoic was large enough to cover the increase in trienoic acid.

Together with the present findings this could mean that during fat deficiency dienoic fatty acid may be mobilized from the depots and, *e. g.* in the liver, be converted into tetraenoic which is then reduced to trienoic. However, it has not yet been proved by direct methods that 5, 8, 11 eicosatrienoic acid originates from dienoic or tetraenoic acid. Thus, the possibility still exists that the trienoic acid of fat deficiency is synthesized in the animal body from oleic acid or saturated acids as has been pointed out by NUNN and SMEDLEY-MACLEAN (1938).

From fig. 4 it is also seen that the concentration of pentaenoic acid in the liver increases with increasing dose of peanut oil until a maximum value of 1.2 mg per g liver is reached when the daily dose of peanut oil is 180 mg/rat.

Hexaenoic and dienoic acid concentrations (not plotted in fig. 4) were practically independent of the daily dose of peanut oil. The concentration of dienoic and hexaenoic acids was approximately 1.0 and 0.4 mg/g liver, respectively.

Comparison of the end points of the curves in fig. 3 and fig. 4 shows that only extremely low tetraenoic and extremely high trienoic liver fatty acids correspond to marked scaliness of the tail, whereas intermediate values for tetra- and trienoic liver fatty acids, such as those found when 38 mg peanut oil are fed, correspond to a low degree of scaliness (below degree 1).

### Summary.

When newly weaned female rats were given (through a period of 183 days) a fat-free basal diet supplemented with 0, 38, 89, 178, and 444 mg peanut oil per day (corresponding to 0, 8, 19, 39, and 97 mg linoleic acid) the following changes were observed:

1. The amount of dienoic acid as percentage of the fat in the fat tissue declined sharply with time during the first 40 days and then levelled off at values nearly proportional to the daily dose of peanut oil.

2. In the liver the following results were obtained at the end of the feeding period:

Expressed as mg per g liver, the amount of dienoic acid was not influenced by the peanut oil supplement.

Trienoic declined with increasing doses of peanut oil. A value almost equal to 0 was obtained with the largest dose of peanut oil fed.

Tetraenoic increased with increasing doses of peanut oil, following a curve which levelled off when the dose of peanut oil was near the maximal dose given.

The sum of trienoic and tetraenoic was not influenced by the amount of peanut oil fed; this phenomenon is discussed in relation to the origin of the trienoic acid of fat-deficiency.

Scaly tail developed in the beginning of the feeding period with all doses of peanut oil. This sign declined after 50—90 days and was absent after 140 days of feeding, except when no peanut oil or the lowest dose (38 mg) was given.

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## Further Studies on the Intestinal Glucuronide Synthesis.

By

K. J. V. HARTIALA, P. LEIKKOLA and P. SAVOLA.

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In our previous *in vitro* studies it was found that contrary to the older concepts the glucuronide synthesis was carried out not only by the liver and kidney but also by the mucous membrane of the whole gastrointestinal tract (HARTIALA 1954 and 1955). These observations were made by using o-aminophenol as the detoxified agent and applying tissue slices using the Warburg-incubation techniques developed for this purpose (LEVY and STOREY 1949, HARTIALA and RONTU 1955).

In order to obtain information about the same synthesis in the whole animal, experiments were performed in which the glucuronide formation was studied after feeding the animal suitable amounts of cinchophen. Previous studies have shown that cinchophen is excreted in the form of glucuronide in the urine (MAGEE, KIM and IVY 1951). The ultimate purpose of these studies was to find whether cinchophen also belongs to substances conjugated to glucuronide in the gastrointestinal tract and whether this synthesis really takes place in the whole animal. This kind of information was desired since in connection with studies on the mechanism of the cinchophen ulcer a hypothesis was put forward (HARTIALA 1955) that the depletion of mucous secretion by the duodenal glands observed during cinchophen intoxication was due to the local glucuronide binding function of the duodenal mucous membrane. The exhaustion of mucous secretion could according to this postulate be a consequence of this double function of the mucous secreting elements in the mucous membrane. As is known also the mucoprotein material produced by this area contains glucuronides.

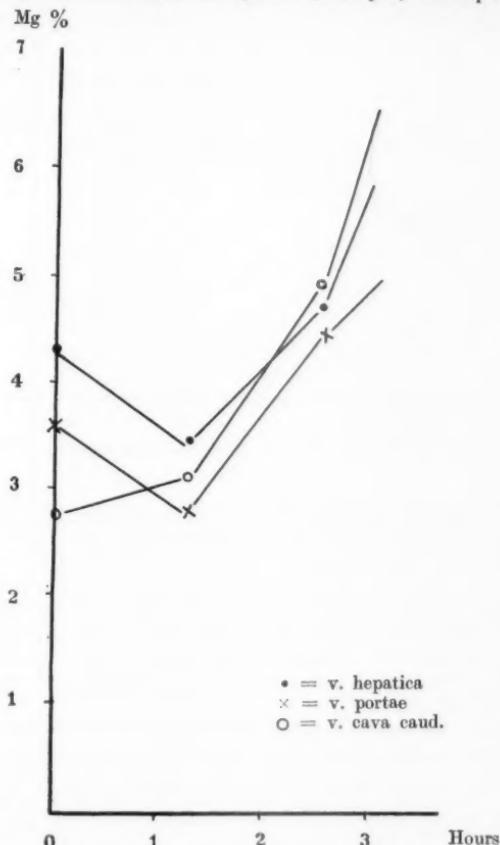
*Serum glucuronides after intragastric feeding of cinchophen.*

Diagram 1. Dog I. 3 g cinchophen administered at 0 hours.

**Material and Methods.**

Mongrel dogs were used in the experiments. They were anesthetized with sodium pentobarbital (Nembutal) given intraperitoneally. Constant respiration rate was maintained by a respiration pump.

After exposure of the major abdominal blood vessels through a midline excision in the abdominal wall, blood samples could be collected directly from the portal veins, the vena hepatica as well as vena cava inferior. In the latter part of the work samples from the femoral vein were also taken.

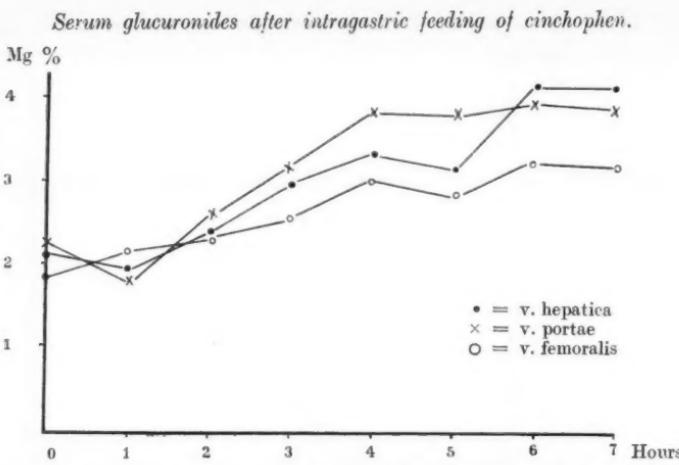


Diagram 2. Dog II. Treated with 500 mg cinchopen at 0 hours.

Cinchopen in amounts stated in the graphs, was given through a gastric tube directly into the stomach. It was mixed into tragacantha solution. The total amount of liquid introduced to the stomach was 50 ml.

Blood samples were taken before the administration of cinchopen and thereafter hourly. The blood was drawn into syringes containing minute amounts of concentrated sodium citrate solution. The blood samples were deproteinized with 10% sodium wolframate solution in 0.60 N sulfuric acid.

The glucuronide determinations were performed immediately thereafter. The modification of the naphthoresorcinol reaction introduced by FISHMAN and GREEN (1955) was applied. The suitability of this method for blood glucuronide determinations was also critically checked by us by adding weighed amounts of glucuronide acid lactone or phenolphthalein-glucuronide into blood. The recovery of these glucuronide compounds by this method proved to be very satisfactory.

### Results and Conclusions.

Fig. 1 represents the results obtained after feeding 3 g of cinchopen into the stomach. It appeared that the glucuronide content in all of the tested parts of circulation increased in about 1 hour after the cinchopen feeding. At the time of 3 hours after the administration of cinchopen the rise was still continuing. At this time the portal blood glucuronide content was some 40

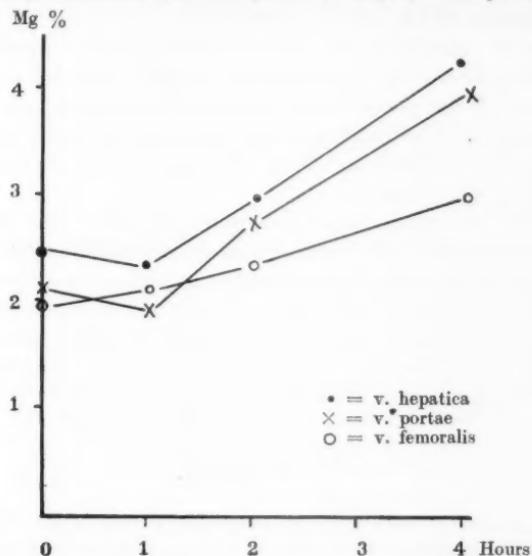
*Serum glucuronides after intragastric feeding of cinchophen.*

Diagram 3. Dog III. D. choledochus ligated. Treated with 500 mg cinchopen at 0 hours.

per cent higher than the previous value. The observed high glucuronide value in the vena cava inferior blood was thought to be due to the high dose of cinchophen used and this perhaps exceeding the capacity of the kidney to eliminate the conjugate.

Experiments were then continued with smaller doses of cinchophen. Fig. 2 shows the result using 500 mg of cinchophen. Here again a definite rise also in the portal venous blood glucuronide content was present; the values at 4 hours had increased 100 per cent from the pre-treatment values. With this dose the femoral vein contained less glucuronide than the hepatic or portal veins.

The rise of glucuronide content in the portal blood need not yet necessarily mean an active synthesis of glucuronide in the gastrointestinal tract. The possibility still existed that the glucuronide in the portal blood could be synthesized in the liver, excreted with the bile into the intestine and after reabsorption cause an increased glucuronide level in the portal blood. It was

considered necessary to eliminate this possibility by preventing the passage of bile into the intestine.

Fig. 3 illustrates the results obtained after ligation of the common bile duct. Interestingly enough, this did not prevent the rise in the portal blood glucuronide contents; now however, the hepatic blood had higher glucuronide concentrations than the portal blood. Analyses of the bile content also revealed a high glucuronide content.

These studies may be taken as an indication that the feeding of cinchophen leads to an increased glucuronide content of the blood coming from the intestine. In accordance with our previous *in vitro* studies this also indicates that the gastrointestinal tract itself is capable to perform glucuronide synthesis and that this mechanism serves as a part in the whole detoxication and absorption mechanism of the organism.

### Summary.

Intragastric feeding of cinchophen in dog results in a significant increase in the portal blood glucuronide concentration in addition to the rise in hepatic and femoral blood glucuronide contents. Ligation of the common bile duct does not prevent the increase in portal blood glucuronide concentration.

These observations are taken as a further evidence to support our *in vitro* studies with tissue slices. These have shown that the mucous membrane of the gastrointestinal tract participates in the detoxication mechanisms and is capable to conjugate glucuronides with suitable substances present in the environment.

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## On the Toxicity of Sodium $\beta$ -mercaptopropruvate and Its Antidotal Effect against Cyanide.

By

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Received 19 October 1957.

Beta-mercaptopropruvic acid and its sodium salt were first synthesized by PARROD (1942, 1947). This compound has been shown to be an intermediate in the cysteine metabolism being formed by transamination of the latter (MEISTER, FRASER and TICE 1954).

It has been reported that salts of  $\beta$ -mercaptopropruvic acid (MPA) are effective in the treatment of experimental liver damage caused by carbon tetrachloride (PARROD, LOEPER and LOEPER 1952, LOEPER and LOEPER 1952 a and b). However, no results in human cases have been reported, possibly because no data are available concerning the toxicity of MPA. This prompted us to try to determine the acute toxicity of MPA in form of its sodium salt.

MPA has also been reported to be effective in reducing the X-ray sensitivity of *Escherichia coli* (BURNETT, STAPLETON, MORSE and HOPPNER 1951, HOPPNER, BAKER and ANDERSON 1951) but was found to have no protective effect in mice (PATT, MAYER and SMITH 1951). This has also been confirmed in our laboratory (CLEMEDSON, HOLMBERG, NELSON and SÖRBO 1956).

It has been found that the sulfur of MPA is transferred to cyanide giving thiocyanate, a transsulfurase catalysed reaction

(WOOD and FIEDLER 1953, SÖRBO 1954), and this suggested that MPA could have an antidotal effect in cyanide poisoning.

Finally the possible cholinergic effect of the compound was investigated. In a recent paper by BUSCH, MARTIN, NYHAN and ZARATZIAN (1956) a series of  $\beta$ -substituted derivatives of pyruvic acid were reported to have such an effect in the intact animal. This effect was attributed by them to a formation of substituted acetylcholines from the pyruvic acid derivative. The structure of MPA suggests that a similar effect might be given by this compound.

### Materials.

Sodium  $\beta$ -mercaptopyruvate was synthesized according to PARROD (1947) from  $\beta$ -chloropyruvic acid and sodium hydro-sulfide. The chloropyruvic acid was synthesized from sulfuryl chloride and pyruvic acid (STEKOL 1948), and the crude product was used without distillation. The yield of mercaptopyruvate was 57 per cent calculated on the pyruvic acid.

The substance must be kept under dry conditions, or in other case recrystallized frequently in order to eliminate the highly toxic hydrogen sulfide formed in the presence of humidity.

### Methods.

*Acute toxicity.* The acute toxicity of the compound was tested by intraperitoneal injection in albino mice weighing about 20 grams. The period of observation was 24 hours.

*Experiments in the anaesthetized cat.* The animals were anaesthetized with sodium pentobarbital (30 mg per kg body weight) administered intraperitoneally. The blood pressure was recorded on a smoked drum by means of a mercury manometer connected to the right common carotid artery. The respiration was recorded simultaneously with the aid of a pneumograph. The anterior tibial muscle was used for investigating the possible effects on neuromuscular transmission. The stimulation of its nerve was supramaximal and repeated every 5 sec.

*Experiments on the isolated rabbit ileum.* The organ bath had a capacity of 100 ml. Ringer solution was used, and the temperature was 33° C.

*Antidote Experiments.* For the study of the effect of MPA as an antidote against hydrocyanic acid poisoning the cyanide was administered as HCN. 125 mg of KCN and 19.2 ml 0.1 N HCl was made up to 50 ml with normal saline. This solution contained 1.0 mg CN<sup>-</sup> per ml.

The antidote experiments were performed in rats and rabbits. In the rabbits the MPA was injected intravenously in doses of 200 mg and 500 mg per kg body weight in one of the marginal ear veins. Immediately afterwards the cyanide was injected into the same vein. The duration of the cyanide injection was kept constant at 30 sec.

In the rats the intraperitoneal route of administration of the cyanide and the antidote was chosen and the dose was 500 mg per kg body weight. In these experiments there was a time interval of 15 min. between the injection of MPA and that of cyanide.

### Results.

*Toxicity and general pharmacology.*  $\beta$ -mercaptopropyruvic acid had a very low toxicity, and the limited solubility of the compound prevented exact determination of the acute toxicity and other pharmacological effects.

In mice lethal effect was sometimes obtained in doses exceeding 3.5 g per kg body weight. These animals died several hours after the injection, and they showed no other symptoms than slight convulsions and respiratory distress.

In the unanaesthetized rabbit doses up to 1.5 g per kg body weight were given intravenously without any symptoms whatsoever.

In the anaesthetized cat only the highest doses given (1.0–1.5 g per kg body weight) resulted in a slight depression of the blood pressure. This effect was slow in onset, and the blood-pressure returned slowly to normal. The blood-pressure effect was unaltered by atropine, given intravenously. MPA gave no potentiation of the blood-pressure response obtained with intravenously given acetylcholine, and it had no visible effects on respiration and neuromuscular transmission in the doses given above.

On the isolated rabbit ileum MPA had no certain effects in concentrations up to 5 mg per ml in the organ bath.

*Antidote Experiments.* In a few preliminary experiments the effect of MPA on the toxicity of intravenously injected cyanide in rabbits was tested. The effect was, however, found to be only very small. Thus, of 5 animals which had been given 200 mg of MPA per kg body weight immediately before a rapid injection of 1.5 mg cyanide per kg body weight 2 died and the other 3 were heavily intoxicated but survived. A dose of 2.5 mg cyanide per kg body weight given to 5 rabbits which had immediately before received a protective dose of 500 mg MPA per kg body weight rapidly killed all the animals. The LD<sub>50</sub> for cyanide administered intravenously to rabbits has been found earlier by CLEMEDSON, HULTMAN and SÖRBO (1954) to be about 0.7 mg HCN per kg body weight.

The toxicity of cyanide given by intraperitoneal injection to rats was determined in a series of 97 animals according to the method described by MILLER and TAINTER (1944). The LD<sub>50</sub> was found to be  $3.1 \pm 0.3$  mg per kg body weight.

When MPA was given 15 min. before the cyanide injection a significant increase in the LD<sub>50</sub> was obtained. The LD<sub>50</sub> was now about 13.5 mg per kg body weight; thus, the compound protected the animals against about 4 lethal doses.

### Discussion.

If MPA is prepared and stored under such conditions that hydrogen sulfide is not formed, the compound has a low toxicity and it seems reasonably safe to use it in treatment of liver disorders, as suggested by PARROD (1952).

The results obtained in the present investigation suggest that MPA has no or considerably weaker cholinergic effects than the substituted pyruvic acids, described by BUSCH, MARTIN, NYHAN and ZARATZIAN (1956). No explanation of this phenomenon can be given.

The considerably less protective effect of MPA against hydrogen cyanide in the rabbit than in the rat may be explained by the fact that rabbit blood shows a much lower transsulfurase activity than rat blood. As the activity of this enzyme in human blood is also lower than in the rat although somewhat higher than in the rabbit (LJUNGGREN and SÖRBO 1957), the antidote effect of MPA in human cases of cyanide poisoning may be limited.

### Summary.

1. Sodium  $\beta$ -mercaptopyravate (MPA) was found to have a low toxicity when injected in different animals.
2. The compound had no or only weak cholinergic effects.
3. The antidote effect against hydrogen cyanide was low in the rabbit, but in the rat MPA gave protection against about 4 lethal doses of hydrogen cyanide.
4. The difference in the antidote effect of MPA in the rabbit and the rat is briefly discussed.

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## **Blood Volume in Albino Rats Determined by the Plasma Dye Method.**

By

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Received 28 October 1957.

The increasing use of albino rats for medical investigation especially in pharmacological and haematological experiments has made it desirable to obtain an average value for the blood volume of rats in proportion to bodyweight.

Only a limited number of investigations have been carried out in this field using modern valid technique *i. e.* the plasma dye method with Evans dye or radioactive tracer technique. The mean values thus obtained have a wide range of variations. (BERLIN, HUFF, VAN DYKE and HENNESSY (1949):  $4.59 \pm 0.57$  ml/100 g in 12 animals, and BECKWITH and CHANUTIN (1941):  $7.98 \pm 0.85$  ml/100 g in 53 animals).

In the present investigation, the most simple technique has been employed to obtain a dependable average value for discussion, rather than making attempts to find and use methods, by which the animals survive, however useful these might be for individual determinations.

### Method.

Normal adult albino rats from an inbred laboratory strain<sup>1</sup> were used for the experiments. Food was withheld for about twenty-four hours before the determinations; water was given without restriction. After ether anaesthesia, a midline laparotomy was performed. The bleeding by this procedure was negligible. 0.3 ml of 0.3 % (weight per volume) Evans dye solution was slowly injected in the easily visualized inferior caval vein, with the point of the needle visible through the venous wall at least at the beginning of the injection. The needle was left in place half a minute after the injection was completed. Syringe and needle were carefully weighed before and after the injection to evaluate the exact amount of dye injected. The blood samples for determination of dye dilution were taken through a cannula from the abdominal aorta four minutes after the injection. Only the first two or three ml of blood withdrawn in a few seconds were kept for analysis and haematocrit determination, although much larger amounts of blood could have been obtained. Clotting was prevented by the injection of 0.3 ml of a 5 % heparin solution into the caval vein immediately prior to the puncture of the abdominal aorta. Direct addition of dry heparin or small quantities of heparin solution to the centrifuge tubes causes an unpredictable degree of opalescence, very deleterious for the photometric determination of the dye concentration. After withdrawal of a sufficient amount of blood for haematocrit determination, the rest of the blood was centrifuged at 3,500 RPM for half an hour. Before the photometric determination, the samples were inspected and all samples with visible haemolysis discarded.

Unicam (mod. S. P. 600) spectro-photometer was used for determination of the optical density at wave-length 620 m $\mu$ . A small specially made cuvette containing 0.25 ml plasma, with a 4 mm light path-way fitted in an adapter to let all the light from the slit pass through the sample, was used for the reading. Zero-setting by dye free plasma and dark current of the photocell were controlled before and after every reading. Plasma dye concentrations were estimated by comparison with two standards made up by carefully weighed amounts of dye free plasma and the dye solution used for the injection. After the method of dye con-

<sup>1</sup> The rats were kindly furnished by Leo Pharmaceutical Products, Copenhagen.

centration determination prior to the proper experiments had been practised for some time, the inaccuracy of 10 consecutive determinations ranged from 0.5 % to 5.0 % (mean: 1.9 %).

The blood volume was calculated from the injected quantity of dye, the plasma concentration and the haematocrit, which is reduced by 5 % for "trapped plasma". Finally 0.6 ml was subtracted, corresponding to the injected amount of dye- and heparin solutions.

### Results and Discussion.

Thirty-one determinations of the circulating blood volume in 9 male and 22 female rats weighing from 177 g to 318 g ranged between 3.68 ml/100 g and 8.24 ml/100 g. The variation had no significant relation to weight or sex. The average value was  $5.92 \text{ ml/100 g} \pm 0.21 \text{ ml}$ .

The method of WELCKER (1858) and the dye dilution methods using vital red, originally introduced by KEITH and ROWNTREE (1915) offer wellknown systematical errors. The results from the vital red method are less dependable, owing particularly to the inaccuracy of the dye concentration determination.

The findings of METCOFF and FAVOUR (1944) and those of BECKWITH and CHANUTIN (1941) respectively  $7.2 \text{ ml} \pm 1.60/100 \text{ g}$  and  $7.98 \pm 0.85 \text{ ml/100 g}$ , both determined using Evans dye, are higher and the latter significantly higher than the figures found by us, but the method of BECKWITH and CHANUTIN (1941) has several disadvantages (small blood samples (0.02 ml) of "cut tail" blood which makes it necessary to dilute the samples before photometry in order to avoid the influence of the probably inevitable haemolysis), and possibly this may account for the discrepancy. Our values are, on the other hand, higher than the values obtained by LORING (1954) (5.04 ml/100 g). The technique of the latter investigator offers however, in our opinion one moment of hazard: the injection of dye, and the withdrawal of blood samples are performed with the same needle by heart puncture. This may very easily lead to contamination of the blood samples with injection solution, either by adherence of the dye to the needle or the syringe, or by re-aspiration of small amounts of dye deposited in the route of puncture. Such contamination will give too small values for the blood volume. Further, a mixing time of only two minutes is

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perhaps, in some instances, rather short for complete mixing of dye and blood. In our technique, much stress has been laid on the complete separation of injection and the withdrawal of blood samples. Our values are also higher than those of BERLIN, HUFF, VAN DYKE and HENNESSY (1949) (4.59 ml/100 g) who use the radioactive tracer technique. In a survey of the literature BOHR (1954) states, that the results by several authors, who have made comparisons between the Evans' dye method and radioactive tracer technique, are on the average only 7 % higher by the Evans' dye method. We are not able to explain this discrepancy.

The problem of extravascular loss of dye and incomplete mixing is often discussed. According to GIBSON and EVANS (1937), the loss of dye is only 6 % per hour in man, and probably not more in rats.

Consequently, a mixing time of four minutes, as used by us, gives only time for a quite insignificant loss, but time enough for a complete mixing, because of the very rapid circulation in smaller animals.

### Summary.

The average value of 31 determinations of circulating blood volume in adult albino rats by a simple plasma dye method using Evans' dye was found to be  $5.92 \pm 0.21$  ml/100 g body weight with a variation from 3.68 ml/100 g to 8.24 ml/100 g.

This work was aided by a grant from Miss P. A. BRANDT's Foundation.

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## Untersuchungen zur fortlaufenden Durchblutungsregistrierung mit Wärmeleitsonden.

### Beobachtungen an der Skelettmuskulatur der Katze.

Von

**KLAUS GRAF und SUNE ROSELL.**

Eingegangen am 11. November 1957.

Seit GIBBS (1933) das Prinzip des geheizten Thermoelementes für Strömungsmessungen mitteilte, hat eine grosse Zahl von Arbeiten die Ergebnisse fortlaufender Durchblutungsregistrierungen mit Wärmeleitmessern behandelt. Die von GIBBS (1933) beschriebene Anordnung wurde dabei für verschiedene Verwendungszwecke mehrfach modifiziert (SCHMIDT und PIERSON 1934; RICHARDS, WOLF und WOLFF 1942; BENNETT, SWEET und BASSETT 1944; ENGSTRAND 1949; GRAYSON 1952; LUDWIGS 1954; HENSEL 1954; GOLENHOFEN 1956; GRAF, GOLENHOFEN und HENSEL 1957; KIESE und LANGE 1957; DEMLING und GROMOTKA 1957). Eine grosse Zahl von Untersuchungen wurde in den letzten Jahren mit der von HENSEL (1954) angegebenen Modifikation, der zur Durchblutungsmessung innerhalb des Parenchyms konstruierten „Wärmeleitsonde“, durchgeführt, weil hier neben einfacher Handhabung, Anwendbarkeit am Menschen und relativ-quantitativer Auswertbarkeit der Messungen auch die Möglichkeit der fortlaufenden Registrierung gegeben war.

Bei allen Wärmeleitmessern gibt der Wärmeabtransport von einer geheizten Stelle und damit die Wärmeleitzahl des Mediums ein sehr

empfindliches Mass für die Durchblutung. Die Wärmeleitzahl  $\lambda$  (im folgenden angegeben in  $10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$ ) errechnet sich aus

$$\lambda = \frac{K \cdot I^2}{\delta}$$

wobei  $\lambda$  die Wärmeleitzahl des Mediums,  $I$  die Heizstromstärke,  $\delta$  die Übertemperatur der geheizten Lötstelle gegenüber dem Gewebe und  $K$  eine Konstante ist, die für jedes Wärmeleitmessers durch Eichung ermittelt werden muss (näheres bei GRAYSON (1952) und HENSEL, RUEF und GOLENHOFEN (1954)). Wärmeleitfähigkeitsmessungen an durchströmten Geweben haben einen vom Stromzeitvolumen abhängigen höheren Wert als die Wärmeleitzahl des nicht durchbluteten Gewebes. Diese durchblutungsbedingte Wärmeleitzahlerhöhung wird mit  $\Delta\lambda$  bezeichnet und ist das eigentliche Mass für die Durchblutung, welche davon ausgehend in Prozent der Ruhedurchblutung angegeben wird (GOLENHOFEN, HENSEL und HILDEBRANDT 1956). Die Möglichkeit einer absoluten Angabe der Durchblutungsgröße in ml/min oder sogar in ml/min/100 g Gewebe, wie sie GRAYSON (1952) und BIRNIE und GRAYSON (1952) versucht haben, konnte von anderen Autoren (LINZELL 1953, HENSEL, RUEF und GOLENHOFEN 1954; GRAF, GOLENHOFEN und HENSEL 1957; GRAF und STEIN 1957) experimentell nicht gesichert werden. Auch durch postmortale Durchströmung des Gewebes wird die exakte Eichung der  $\Delta\lambda$ -Änderungen in ml/min/100 g Gewebe (KIESE und LANGE 1957) nicht möglich, weil dabei in vivo erfolgende Änderungen der Durchblutungsgröße im maximal etwa 1 cm<sup>3</sup> grossen Messbereich, z. B. durch vasomotorische Reaktionen, nicht berücksichtigt werden können. Von mehreren Autoren wurde übereinstimmend gezeigt, dass sich die Wärmeleitzahl annähernd proportional zur Grösse der Gesamtdurchströmung ändert, so in Durchströmungsversuchen von GIBBS (1933), ENGSTRAND (1949), SCHER (1951), GRAYSON (1952), LINZELL (1953), HENSEL, RUEF und GOLENHOFEN (1954), GRAF, GOLENHOFEN und HENSEL (1957), GRAF und STEIN (1957) und KIESE und LANGE (1957) wie auch im Vergleich mit plethysmografischen (BARCROFT, HENSEL und KITCHIN 1955) und Diathermie-Stromuhr-Untersuchungen (OBERDORF 1954), so dass eine relative Eichung möglich ist.

Unbekannt war noch, welche Abhängigkeit die mit Wärmeleitsonden registrierten Werte von dem am Messort bestehenden Stromzeitvolumen zeigen. Da bei den üblicherweise verwandten Übertemperaturen  $\delta$  von 1.0°—4.0° C der aufgeheizte Gewebsbezirk einen Durchmesser von etwa 10 mm aufweist (CHESTER und GRAYSON 1951), war es ungewiss, ob vorwiegend die Durchblutung grösserer Gefässer wie Arterien und Venen oder auch, in auswertbarem Masse, die Kapillardurchblutung von der Wärmeleitsonde erfasst wird. Gerade diese Frage ist aber für die Auswertung besonders von Muskeldurchblutungsregistrierungen mit

Wärmeleitsonden von Bedeutung. In Experimenten an der Skelettmuskulatur der Katze wurden daher die mit 2 Ausführungen der HENSEL'schen Wärmeleitsonde erzielten Ergebnisse von Durchblutungsmessungen mit den Ergebnissen von gleichzeitig erfolgten direkten Messungen der Muskelgesamtdurchblutung verglichen und die Abhängigkeit der durchblutungsbedingten Wärmeleitzahlerhöhung  $\Delta\lambda$  von den im Messbereich zu findenden Gefässqualitäten untersucht.

### 1. Methodik.

Die Versuche erfolgten an 30 Katzen in Chloralosenarkose (70 mg/kg Chloralose i. v.). Für die blutige Messung des Stromzeitvolumens wurde die Gerinnungsfähigkeit des Blutes durch 25 mg/kg Heparin i. v. aufgehoben. Registriert wurden:

1. die Durchblutung im M. gastrocnemius oder in der Oberschenkel-adductorenmuskulatur mittels der Wärmeleitsonde nach HENSEL (1954).
2. die Gesamtdurchblutung der Muskulatur einer Hinterextremität (s. unten) mittels der Tropfkammermethode nach LINDGREN und UVNÄS (1954). Diese Apparatur war in den venösen Gefässabschnitt eingeschaltet.
3. der arterielle Mitteldruck in der A. carotis communis mittels eines Hg-Manometers.

Alle Messwerte wurden fortlaufend aufgezeichnet:

1. die Wärmeleitzahl über ein Spiegelgalvanometer (Modell Zernike A 14 der Fa. KIPP & Zonen, Delft, Holland) auf einem Direktschreiber (»Nachlaufschreiber« der Fa. Dr. B. LANGE, Berlin). Die Einstellzeit des gesamten Registriersystems für 95 % der Gesamtanzeige betrug 8 sec.
2. die Gesamtdurchblutung der Hinterextremitätenmuskulatur über einen Ordinatenschreiber auf einem Russkymografion, wobei jeder oder jeder 2. Tropfen des venösen Ausflusses von einer Fotozelle registriert und durch den Ordinatenschreiber angezeigt wurde (LINDGREN und UVNÄS 1954). Die Anzeigeverzögerung dieses Systems betrug 2–3 sec (LINDGREN, persönliche Mitteilung).
3. der arterielle Mitteldruck auf einem Russkymografion. Die Differenz von 5 sec in der Einstellzeit für 95 % der Gesamtanzeige der Stromzeitvolumen- und der Wärmeleitzahlmessung konnte für unsere Untersuchungen in Kauf genommen werden.

Um vorwiegend die Durchblutung der Muskulatur des Beines zu erfassen, wurde der Oberschenkel enthäutet (näheres s. LINDGREN und UVNÄS 1954). Eine Ausschaltung der Knochen-, Sehn- und Gelenkdurchblutung war allerdings nicht möglich. Diese ist jedoch mit rund 1 ml/100 g Gewebe/min (EDHOLM, HOWARTH und Mc-

MICHAEL 1945; EDHOLM und HOWARTH 1953; BONNEY, HUGHES und JANUS 1952; COBBOLD und LEWIS 1956) um ein Mehrfaches geringer als die Skelettmuskeldurchblutung (Werte dazu s. Seite 66). Da zudem der Anteil der Knochen und Sehnen am Gesamtvolumen der Extremitäten maximal 20 %, der Anteil der Skelettmuskulatur aber mindestens 60 % beträgt (WHITNEY 1953, COOPER, EDHOLM und MOTTRAM 1955; HATCHER und JENNINGS 1957) kann angenommen werden, dass das registrierte Stromzeitvolumen zum überwiegenden Teil allein durch die Skelettmuskulatur des Beines floss.

Zur Vermeidung stärkerer Unterkühlung wurden beide Hinterextremitäten des Tieres dick mit Watte umhüllt und das ganze Tier mit Rotlicht einer Umgebungstemperatur von etwa 25° C ausgesetzt. Die Rektaltemperatur betrug 35°—39° C.

Bei 10 Versuchen erfolgte die Messung mit der Wärmeleitsonde und der Tropfkammer an der gleichen Extremität. Da es aber oft schwierig war, thermokonstante Sondenlagen zu finden, bei denen Änderungen der Durchblutung keine wesentlichen Änderungen des Temperaturgradienten zwischen den beiden Lötstellen der Wärmeleitsonde bewirkten, wurde bei 16 Versuchen die Wärmeleitsonde schliesslich in die Muskulatur der anderen Hinterextremität eingeführt. Bei 4 Versuchen schliesslich erfolgte die Messung mit brauchbaren Sondenlagen sowohl in der einen wie danach auch in der gegenüberliegenden Extremität.

Änderungen der Muskeldurchblutung wurden mittels elektrischer Reizung der vasodilatatorischen Bahnen im Hypothalamus (LINDGREN, ROSÉN, STRANDBERG und UVNÄS 1956), mittels intravenöser oder intraaortalen Injektionen von Acetylcholin und Adrenalin sowie 3—6-minütiger Drosselung der Hinterextremitätdurchblutung durch Kompression der Aorta abdominalis mit nachfolgender reaktiver Hyperämie hervorgerufen. Auf diese Weise war auch in den Fällen, in denen die Durchblutungsmessung in beiden Hinterextremitäten vorgenommen wurde, ein quantitativer Vergleich der Resultate möglich, da z. B. nach Reizung der Vasodilatatorbahnen im Hypothalamus die Durchblutungszunahmen in der Muskulatur beider Hinterextremitäten quantitativ annähernd gleich gross erfolgen (Uvnäs, persönliche Mitteilung). Gleichermaßen kann auch nach intravenösen und intra-aortalen Injektionen angenommen werden.

Die Auswahl der sogenannten »guten« oder »brauchbaren« Sondenlagen — worunter verstanden wird, dass bei ungeheizter Sonde Durchblutungsänderungen zu keinen wesentlichen Temperaturdifferenzen zwischen den beiden 10 mm voneinander entfernt liegenden Lötstellen des Thermoelementes führen, gleiche Durchblutungsänderungen aber nach Aufheizung der Sondenspitze ausreichend empfindlich angezeigt werden — erfolgte nach den von GOLENHOFEN, HENSEL und HILDEBRANDT (1956) aufgestellten Forderungen. Die Übertemperatur der Sondenspitze gegenüber dem Gewebe betrug 2.0°—4.0° C. Zur Vereinfachung wird im Folgenden die bei Ruhedurchblutung gemessene Wärmeleitzahlerhöhung mit  $\Delta\lambda_R$  bezeichnet. Weitere Einzelheiten der fortlaufenden Durchblutungsregistrierung mit Hilfe der Wärmeleitsonde sind

bereits des öfteren ausführlich beschrieben worden, weshalb zur Technik des Verfahrens auf diese Publikationen verwiesen sei (HENSEL, RUEF und GOLENHOFEN 1954; HENSEL und BOCK 1955; GOLENHOFEN, HENSEL und HILDEBRANDT 1956; GRAF, GOLENHOFEN und HENSEL 1957).

Zur Registrierung der Wärmeleitzahl wurden 2 verschiedene Ausführungen der Wärmeleitsonde verwandt, die sich lediglich durch die Art ihrer Umhüllung (Metall oder Kunststoff) voneinander unterschieden. In der „*Metallsonde*“ (HENSEL 1954) befand sich das Heiz- und Messsystem in einer V2A-Stahlkanüle von 1 mm Aussendurchmesser, die Drähte waren durch feinste Glaskapillaren gegeneinander isoliert. In der „*Kunststoffsonde*“ war das Heiz- und Messsystem an der Spitze auf eine Länge von 2 cm in den starren Kunststoff »Palavit« (Hersteller: KULZER & Co., Bad Homburg, Deutschland) eingebettet. Das Palavite der Sonde war spitz zugefeilt. Der Durchmesser dieser Sondenausführung betrug ebenfalls 1 mm. Das Palavit füllte den gesamten Raum zwischen den Mess- und Heizleitungen aus, wodurch ein bestmöglich Wärmeübergang vom Mess- und Heizsystem auf die Sondenoberfläche erreicht wurde. Eine ähnliche, für Leberdurchblutungsmessungen konstruierte Sondenausführung hatten bereits früher GRAF, GOLENHOFEN und HENSEL (1957) als „*flexible Wärmeleitsonde*“ beschrieben, doch war diese mitsamt der Palavitschicht noch in einen Polyaethylenschlauch eingehüllt. In 19 Versuchen wurde die Metall-Wärmeleitsonde und in 11 Versuchen die Kunststoff-Wärmeleitsonde verwandt.

In der Mehrzahl der Versuche wurde nach dem Tode des Tieres das Muskelgebiet, in dem sich die Wärmeleitsonde befand, sorgfältig präpariert, um durch die Sonde verursachte Gewebsverletzungen und Blutungen und die an der Sondenspitze liegenden Gefäße feststellen zu können. Eine ausreichende makroskopische Erkennung der die Sondenspitze umgebenden Gefäße war jedoch ohne Hilfsmittel nicht möglich. Deshalb wurde in 10 Versuchen (Tab. 1), in denen die Metallsonde verwandt worden war, blaues Vinylacetat in die A. femoralis injiziert und nach Eintritt der Totenstarre die Sonde durch Präparation der Muskulatur freigelegt. Die Gefäße der noch makroskopisch erkennbaren Größenordnung waren nun mit erstarrtem Vinylacetat gefüllt und gut darzustellen. Die fertige Präparation wurde mittels Farbphotographie dokumentiert.

In drei weiteren Versuchen mit der starren Wärmeleitsonde wurden die Extremitätengefäße nach Röntgen-Kontrastmittelinkjection in die A. femoralis fotografiert und die Sonde anschliessend ebenfalls freipräpariert. Als Kontrastmittel wurde »Triurol 70 %« verwandt.

## 2. Ergebnisse.

### a. Auswahl der Sondenlage.

Bei ungeheizter Sonde eine thermokonstante Sondenlage zu finden, hat oft erhebliche Schwierigkeiten bereitet. Als Ursache dafür muss das geringe Volumen der Katzenmuskulatur sowie

der operative Eingriff angesehen werden. Am schnellsten ergaben sich gute Lagen, wenn die Sonde parallel zu grösseren Gefässen verlief, was im allgemeinen einer Sondenlage entsprach, bei der sich beide Lötstellen in annähernd gleicher Tiefe befanden. Ein gutes Beispiel für die Abhängigkeit der Thermokonstanz von der Sondenlage sahen wir in dem Fall, der in Abb. 4 b dargestellt ist. Dort verlaufen mehrere grössere Gefässer teils parallel, teils (an der Sondenspitze und 10 mm sondenaufwärts) quer zur Sonde. Geringes Verschieben der Sonde um nur wenige Millimeter bewirkte hier eine Änderung des Temperaturgradienten bei ungeheizter Sonde um mehrere Zehntel °C. Waren schliesslich thermokonstante Lagen gefunden, so zeigten diese nach durchblutungsändernden Massnahmen häufig nur minimale absolute und prozentuale Änderungen der Wärmeleitzahl, und zwar auch dann, wenn sich die Muskelgesamtdurchblutung gleichzeitig um mehrere hundert Prozent änderte. Darüberhinaus verliefen die Wärmeleitzahländerungen im Vergleich zu den Reaktionen der Muskelgesamtdurchblutung umso träger, je kleiner der  $\Delta\lambda$ -Wert bei Ruhedurchblutung ( $\Delta\lambda_R$ ) war. Gewöhnlich wurde die Sonde solange verschoben, bis die mit beiden Methoden gemessenen Durchblutungsreaktionen in der Form des zeitlichen Ablaufs weitgehend ähnlich waren. Dieses war in zufriedenstellendem Masse (s. unten) erst bei  $\Delta\lambda_R$ -Werten von mindestens  $1.0 \cdot 10^{-4}$   $\text{cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  der Fall.

Die hier beschriebenen Ergebnisse wurden daher zum Teil mit einer Auswahl von bestmöglichen Sondenlagen gewonnen. Im Durchschnitt musste die Sondenlage 10—20 mal bei jedem Versuch verändert werden, ehe sowohl Thermokonstanz mit ungeheizter Sonde wie auch der gewünschte  $\Delta\lambda_R$ -Wert von mindestens  $1.0 \cdot 10^{-4}$  registriert werden konnten. In einer grösseren Zahl von Versuchen gelang dies in der durch die Versuchsbedingungen begrenzten Experimentierzeit von 3—4 Stunden aber nicht; in solchen Fällen wurde der Verlauf von Wärmeleitzahländerungen während stärkerer Änderungen der Gesamtdurchblutung bei geringeren  $\Delta\lambda_R$ -Werten von meist nur  $0.1—0.5 \cdot 10^{-4}$ , in einigen Fällen von  $0.5—1.0 \cdot 10^{-4}$  registriert.

*b. Vergleich von Richtung und zeitlichem Ablauf der Wärmeleitzahl- und Stromzeitvolumenänderungen.*

Einzelinjektionen von Acetylcholin und Adrenalin, die in der blutigen Stromzeitvolumenmessung eine kurzdauernde Durch-

blutungszunahme auf 200—400 % der Ruhedurchblutung erkennen liessen, bewirkten bei  $\Delta\lambda_R$ -Werten unter  $0.5 \cdot 10^{-4}$  cal · cm<sup>-1</sup> · sec<sup>-1</sup> · °C<sup>-1</sup> eine zeitlich stark verzögerte und daher auch prozentual erheblich kleinere Zunahme der Wärmeleitzahl. Erst bei längerem Bestehen der Durchblutungsänderungen, etwas während einer 1—2 min dauernden Infusion von Acetylcholin, erreichte  $\Delta\lambda$  eine prozentuale Zunahme, die der prozentualen Änderung der Gesamtdurchblutung häufig sehr ähnlich war.

Ein typisches Beispiel für dieses Verhalten gibt Abb.1.  $\Delta\lambda_R$  betrug hier  $0.2 \cdot 10^{-4}$ . Bei (1) erfolgte nach einer intra-aortalen Einzelinjektion von 0.1  $\mu\text{g}$  Acetylcholin ein steiler Anstieg der Muskelgesamtdurchblutung auf maximal 400 % des Ruhewertes, der aber bereits nach 15 sec wieder zurückging. Die von der Wärmeleitsonde (hier Kunststoffsonde) registrierte Durchblutungsänderung setzte praktisch synchron mit der Änderung der Gesamtdurchblutung ein, zeigte aber einen nur langsam erfolgenden Durchblutungsanstieg auf maximal 160 % der Ruhedurchblutung, der — wiederum auch nur langsamer als die Änderung der Gesamtdurchblutung — dann ebenfalls abfiel. Bei (2) reagierte die Gesamtdurchblutung während einer 100 sec dauernden Infusion von insgesamt 6  $\mu\text{g}$  Acetylcholin gleichfalls mit einem steilen Anstieg auf etwa 400 % des Ruhewertes. Diese Mehrdurchblutung wurde während der gesamten Infusionsdauer im Mittel gehalten. Die Wärmeleitzahl erreichte erst 90 sec später als die Gesamtdurchblutung ihren Endwert, der in diesem Falle aber von ähnlicher prozentualer Grösse wie die prozentuale Änderung der Gesamtdurchblutung war, obwohl die Muskelgesamtdurchblutung hier nicht stärker als bei (1) anstieg. Nach Beendigung der Infusion war der Rückgang der Wärmeleitzahl gegenüber dem Rückgang der Gesamtdurchblutung wieder stark verzögert.

Mit zunehmend grösseren  $\Delta\lambda_R$ -Werten wurde die Trägheit der Wärmeleitmessung immer geringer. Deutlich war dies bereits bei  $\Delta\lambda_R$ -Werten zwischen  $0.5$ — $1.0 \cdot 10^{-4}$  erkennbar. Bei  $\Delta\lambda_R$ -Werten grösser als  $1.0 \cdot 10^{-4}$  war bei pharmakologischen oder durch Nervenreizung bedingten Durchblutungsänderungen eine wesentliche zeitliche Verzögerung der Wärmeleitmessung nicht mehr zu beobachten.

Ein typisches Beispiel dafür zeigt Abb. 2. In diesem (mit der Metall-Wärmeleitsonde durchgeföhrten) Versuch, in dem  $\Delta\lambda_R = 1.6 \cdot 10^{-4}$  betrug, erfolgte die Mehrdurchblutung der Muskulatur

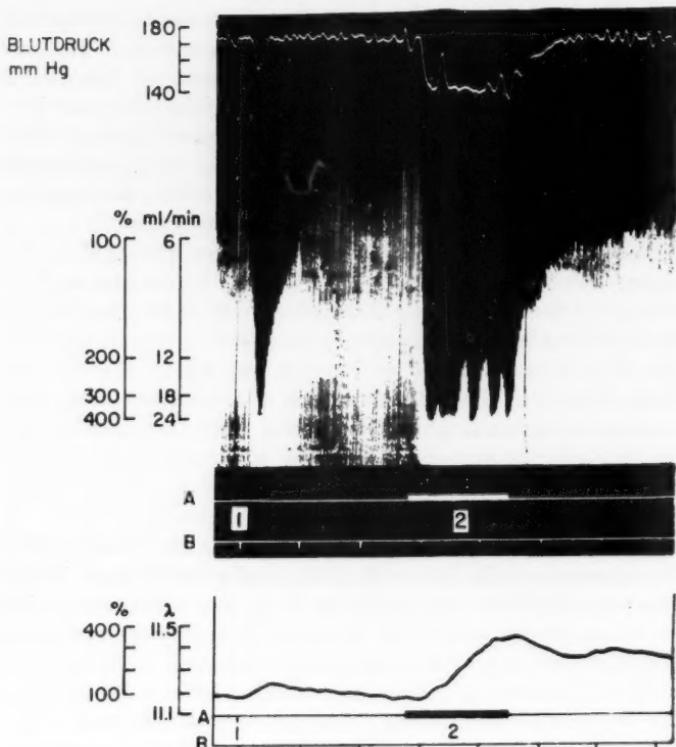


Abb. 1. Verhalten von arteriellem Mitteldruck (oben), Gesamtstromzeitvolumen der Muskulatur des rechten Hinterbeines (mitte) und der Wärmeleitzahl  $\lambda$  im rechten M. gastrocnemius (unten) während intraaortaler Einzelinjektion von 0.1  $\mu$ g Acetylcholin (1) und 100 sec dauernder intraaortaler Infusion von 6  $\mu$ g Acetylcholin (2).

Durchblutungsangaben zusätzlich in Prozent der Ruhedurchblutung. Wärmeleitmessung mit der Kunststoff-Wärmeleitsonde.

A = Injektions- und Infusionsmarkierung.

B = Zeiteichung in Minuten.

nach Reizung der vasodilatatorischen Bahnen im Hypothalamus. Die Wärmeleitzahländerung verlief praktisch synchron zu den Änderungen der Gesamtdurchblutung, und auch die mit den beiden Methoden gemessenen prozentualen Wärmeleitzahländerungen waren von ähnlicher Grösse.

In allen Fällen war die Richtung von Stromzeitvolumenänderungen und Wärmeleitzahländerungen gleich.

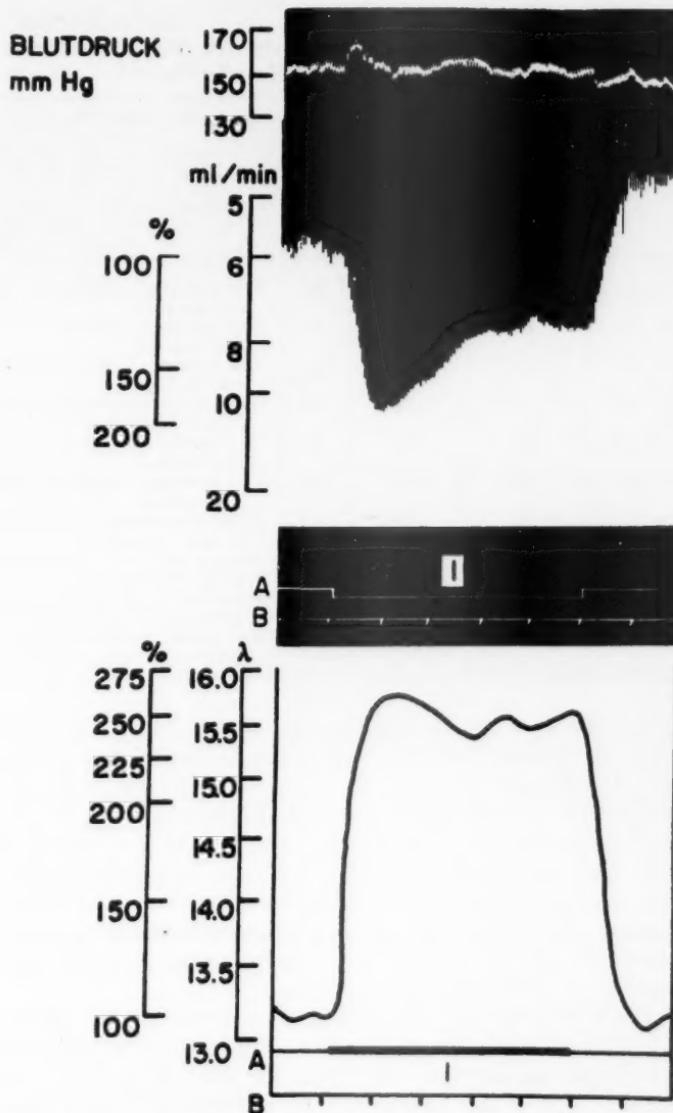


Abb. 2. Verhalten von arteriellem Mitteldruck (oben), Gesamtstromzeitvolumen der Muskulatur des rechten Hinterbeines (mitte) und der Wärmeleitzahl  $\lambda$  im linken M. gastrocnemius (unten) während elektrischer Reizung der hypothalamischen Vasodilatatorzentren (I). Durchblutungsangaben zusätzlich in Prozent der Ruhedurchblutung.

Wärmeleitmessung mit der Metall-Wärmeleitsonde.

A = Reizmarkierung.  
B = Zeiteichung in Minuten.

Tabelle 1.

Beziehung zwischen Wärmeleitzahlerhöhung bei Ruhedurchblutung ( $\Delta\lambda_R$ ) und Lage der Sondenspitze (Metallsonde).

Vers. Nr.	$\lambda$ Nichtdurch- bluteter Muskel	$\lambda$ Ruhedurch- blutung	$\Delta\lambda_R$	Lage der Sondenspitze
				(alle Werte in $10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$ )
1 .....	11.9	12.4	0.5	Von sichtbaren Gefässen 5—6 mm entfernt.
2 .....	11.6	12.1	0.5	Von sichtbaren Gefässen 2—3 mm entfernt
3 .....	10.8	11.3	0.5	Von sichtbaren Gefässen 5—6 mm entfernt
4 .....	11.4	12.0	0.6	Im Muskelparenchym. Sichtbare Gefässe 5 mm entfernt
5 .....	11.3	12.0	0.7	Von sichtbaren Gefässen 5—6 mm entfernt
6 .....	11.6	12.8	1.2	Von sichtbaren Gefässen 4—5 mm entfernt
7 .....	9.2	10.6	1.4	In der Fossa poplitea direkt an A. und V. poplitea
8 .....	11.6	13.2	1.6	Arterien und Venen unmittelbar an der Sondenspitze
9 .....	10.8	13.0	2.2	Direkt an u. parallel mit grösserer Arterie verlaufend
10 .....	11.7	15.3	3.6	Von grossen Arterien u. Venen unmittelbar umgeben

Von den 10 in Tab. 1 aufgeführten, mit der Metall-Wärmeleitsonde angestellten Versuchen zeigten nur 5 (Nr. 6, 7, 8, 9 und 10) mit  $\Delta\lambda_R$ -Werten von mehr als  $1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  eine weitgehende zeitliche und prozentuale Übereinstimmung mit den Änderungen der Muskelgesamtdurchblutung. In den anderen 5 Versuchen mit  $\Delta\lambda_R$ -Werten von  $0.5—0.7 \cdot 10^{-4}$  konnten zwar ebenfalls gleichgrosse Änderungen der Gesamtdurchblutung beobachtet werden, doch waren hier die Wärmeleitzahländerungen zeitlich stark verzögert und daher bei kurzdauernden Durchblutungsreaktionen prozentual nur sehr klein.

Die 5 Versuche mit schnellen und prozentual grossen Wärmeleitzahländerungen hatten sämtlich einen  $\Delta\lambda_R$ -Wert von mehr als  $1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  (1.2; 1.4; 1.6; 2.2;  $3.6 \cdot 10^{-4}$ , Versuche 6, 7, 8, 9 und 10 aus Tab. 1), während die trägen Reaktionen ausgingen von  $\Delta\lambda_R$ -Werten unter  $0.7 \cdot 10^{-4}$  (0.5; 0.5; 0.5; 0.6 und  $0.7 \cdot 10^{-4}$ , Versuche 1—5 aus Tab. 1).

Bei der Kunststoff-Wärmeleitsonde war, wie wir regelmässig

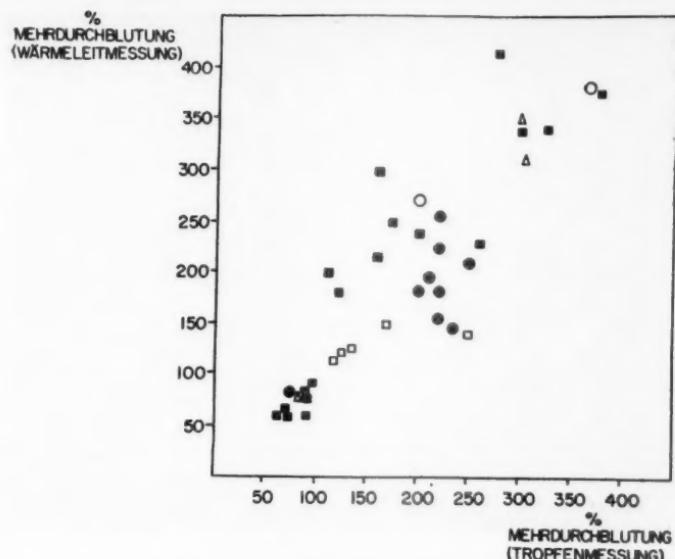


Abb. 3. Zunahme der Wärmeleitzahl im linken M. gastrocnemius in Abhängigkeit von der Zunahme des Gesamtstromzeitvolumens der Muskulatur des rechten Beines nach i. v. und intraaortalen Injektionen und Infusionen von Acetylcholin und Adrenalin sowie nach elektrischer Reizung der hypothalamischen Vasodilatatorbahnen. Werte in Prozent der Ruhedurchblutung. (● = Vers. Nr.: 6, □ = Vers. Nr.: 7, ○ = Vers. Nr.: 8, ■ = Vers. Nr.: 9, △ = Vers. Nr.: 10 aus Tabelle 1).

beobachten konnten, die zeitliche Verzögerung etwas grösser als bei der Metall-Wärmeleitsonde. Eine quantitative Angabe dieses Unterschiedes erlauben die bisher vorliegenden Versuchsergebnisse noch nicht. Der Unterschied ist aber nicht so gross, als dass die Messungen mit den beiden verschiedenen Sondenausführungen nicht gemeinsam besprochen werden könnten.

*c. Quantitativer Vergleich der Ergebnisse von Stromzeitvolumen- und Wärmeleitzahlmessungen.*

Abb. 3 zeigt aus den 5 Versuchen der Tab. 1 mit »guten« Sondenlagen ( $A\lambda_R$  mindestens  $1.0 \cdot 10^{-4}$ ) die prozentualen Zunahmen der Wärmeleitfähigkeit in Abhängigkeit von der prozentualen Steigerung der Gesamtdurchblutung der Muskulatur nach intraaortalen Injektionen und Infusionen von Acetylcholin und Adrenalin und elektrischer Reizung der vasodilatatorischen Bahnen im Hypothalamus. In allen diesen Versuchen wurde die



Abb. 4 a.

$$\Delta\lambda_R = 0.6 \cdot 10^{-4}$$

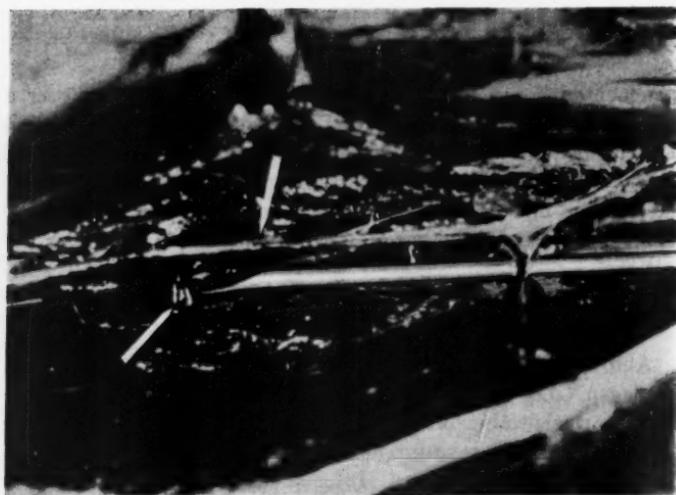


Abb. 4 b.

$$\Delta\lambda_R = 1.6 \cdot 10^{-4}$$



Abb. 4 c.

$$\Delta\lambda_R = 2.2 \cdot 10^{-4}$$

Abb. 4 a—c. Postmortale Präparationen des *M. gastrocnemius*. Bilder aus 3 verschiedenen Versuchen (Kopien von Farb-Diapositiven). Man erkennt deutlich die von rechts in das Bild hineinragende Metall-Wärmeleitsonde, deren Durchmesser 1 mm beträgt. Die hellen Pfeile bezeichnen die mit Vinylacetat gefüllten makroskopisch erkennbaren Gefäße.

Muskeldurchblutung mit der Tropfammermethode im rechten und mit der Wärmeleitsonde im linken *M. gastrocnemius* gemessen.

Das Ergebnis zeigt, dass bei einem  $\Delta\lambda_R$ -Wert von mindestens  $1.0 \cdot 10^{-4}$  auch bei schnelleren Durchblutungsänderungen die prozentualen Wärmeleitzahländerungen den prozentualen Änderungen der Gesamtdurchblutung der Muskulatur sehr ähnlich sind. In keinem Falle war die Abweichung von der direkten Proportionalität zwischen beiden Messergebnissen grösser als 50 % des grösseren gemessenen Wertes. Bei allen anderen Messungen aber, die von einem  $\Delta\lambda_R$ -Wert von nur  $0.6 \cdot 10^{-4}$  oder weniger ausgingen, waren die relativen Gesamtdurchblutungsänderungen oft um 80—90 % grösser als die relativen Änderungen von  $\Delta\lambda$ .

#### *d. Postmortale Präparation der Sondenlage.*

Bei den in Tab. 1 aufgeführten 10 Versuchen wurde nach Eintreten der Totenstarre die Muskulatur präpariert und die Lage der

Sondenspitze in Beziehung zu den sie umgebenden makroskopisch sichtbaren Gefäßen untersucht (näheres s. Methodik). In allen Fällen, in denen wohl die Gesamtdurchblutung, nicht aber die Wärmeleitzahl stärkere Durchblutungsreaktionen anzeigte, betrug der Abstand zwischen der Sondenspitze und den sichtbaren Gefäßen stets mehr als 5 mm. Je näher sich die Gefäße, ausgehend von einer maximalen Entfernung von 5—6 mm, der Sondenspitze befanden und je grösser das Kaliber dieser Gefäße war, umso grösser war  $\Delta\lambda_R$  und umso mehr stimmten die Wärmeleitzahländerungen mit den Änderungen der Gesamtdurchblutung überein.

Die Abb. 4 a—c sollen dieses veranschaulichen. Die mit Vinylacetat gefüllten makroskopisch sichtbaren Gefäße sind durch helle Pfeile bezeichnet. Deutlich ist die stets von rechts in das Bild eintretende Metall-Wärmeleitsonde zu erkennen, deren Durchmesser 1 mm beträgt und damit den Massstab für die räumlichen Verhältnisse darstellt. In reinem Muskelparenchym (Abb. 4 a) in dem nur im Abstand von 3 mm und mehr von der Sondenspitze grössere Gefäße lagen, waren lediglich  $\Delta\lambda_R$ -Werte von  $0.1 - 0.7 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  zu messen (Abb. 4 a:  $\Delta\lambda_R = 0.6 \cdot 10^{-4}$ ). Die hohen  $\Delta\lambda_R$ -Werte von mehr als  $1.0 \cdot 10^{-4}$  sahen wir nur bei Sondenlagen, bei denen entweder die Sondenspitze ein grosses Gefäß bzw. auch mehrere berührte (Abb. 4 b:  $\Delta\lambda_R = 1.6 \cdot 10^{-4}$ ) oder solche sehr grossen Gefäße parallel und in unmittelbarem Kontakt mit den vordersten 1—2 cm der Sonde verliefen (Abb. 4 c:  $\Delta\lambda_R = 2.2 \cdot 10^{-4}$ ).

Die Versuche 2 und 6 in Tab. 1 fallen aus dem Rahmen des eben Gesagten heraus. Beim Versuch 6 war  $\Delta\lambda_R$  verhältnismässig gross im Vergleich zu der Entfernung der Gefäße von der Sondenspitze. Beim Versuch 2 ist das Umgekehrte der Fall, hier war  $\Delta\lambda_R$  auffallend klein im Vergleich zum Abstand der Gefäße von der Sondenspitze. Bei beiden Versuchen sahen wir, dass sich die Wärmeleitsonde während der Muskelpräparation um einige Millimeter verschob. Dies kann wohl als Erklärung für die Differenzen angesehen werden.

Makroskopisch erkennbare *Blutungen* in der Muskulatur als offensichtliche Folge des Sondeneinstichs wurden in 3 Versuchen beobachtet. Einmal war eine Blutung nur an der Oberfläche des M. gastrocnemius in einer Ausdehnung von 2—3  $\text{cm}^2$  erkennbar, in den beiden anderen Fällen breiteten sich Blutungen in einer Länge von 1—2 cm in intermuskulären Septen, welche grössere

Gefässen enthielten, aus. Kleinere punktförmige Blutungen im Muskelparenchym wurden nie gesehen. Da in sämtlichen 30 Versuchen zusammen die Sonde wenigstens 300mal in die Muskulatur eingestochen wurde, hat annäherungsweise also die Wärmeleitsonde nur in 1 % der Fälle stärkere Gewebsverletzungen mit Blutungen verursacht.

### 3. Diskussion.

Die Untersuchungen haben ergeben, dass die Grösse des konvektiven Wärmeabtransports im Skeletmuskel für eine genügend empfindliche und frequente Durchblutungsmessung nur dann ausreichend ist, wenn der aufgeheizte Gewebsbezirk von makroskopisch sichtbaren Gefässen durchzogen wird. Wie CHESTER und GRAYSON (1951) gezeigt haben, besitzt der aufgeheizte Gewebsbezirk bei einer Übertemperatur von 1 °C einen Radius von maximal 5 mm. Dabei soll aber der Hauptteil der Messung in einem wesentlich kleineren Gebiet stattfinden, da  $\delta$  mit zunehmender Entfernung vom Heizort anfangs besonders steil abfällt und 1—2 mm vom Heizort entfernt nur noch 50 % des Ausgangswertes beträgt.

Diese Ergebnisse von CHESTER und GRAYSON (1951) stehen mit unseren Befunden in Einklang. Höhere  $\Delta\lambda_R$ -Werte von mehr als  $1.0 \cdot 10^{-4}$  cal·cm $^{-1}$ ·sec $^{-1}$ ·°C $^{-1}$  mit genügend frequenter und empfindlicher Durchblutungsanzeige waren nur dann zu messen, wenn grössere Gefässen im Abstand von höchstens 2—5 mm von der Sondenspitze lagen, was annäherungsweise dem Radius des aufgeheizten Bereiches entspricht. Befand sich die Sondenspitze dagegen in reinem, nicht von grösseren Gefässen durchzogenem Muskelparenchym oder in einem Bindegewebsseptum, so waren bei Ruhedurchblutung lediglich kleine  $\Delta\lambda_R$ -Werte von 0.1— $0.6 \cdot 10^{-4}$  mit sehr träger Anzeige von Durchblutungsänderungen messbar. In der Skeletmuskulatur kann mit der Wärmeleitsonde folglich direkt nur die Durchblutung von makroskopisch sichtbaren Gefässen, also von Arterien und Venen, in zufriedenstellendem Masse angezeigt werden, nicht dagegen die Kapillarströmung. Diese an der Katze gemachten Beobachtungen können auch auf die Verhältnisse beim Hund oder beim Menschen bezogen werden, weil bei allen diesen Spezies die Skeletmuskeldurchblutung, auf die Gewichtseinheit bezogen, ähnlich gross ist (s. Seite 66).

Natürlich wird die Wärmeleitmessung auch von der Kapillarströmung beeinflusst. Deren konvektiver Wärmeabtransport ist aber sehr gering. Infolgedessen könnten hier Durchblutungsänderungen häufig nicht von den auch bei guten Sonderlagen unvermeidlichen geringen Änderungen des zwischen den beiden Thermoelementen herrschenden Temperaturgradienten  $\delta_\theta$  unterschieden werden, der durch Differenzen in der Gewebstemperatur bedingt ist (s. dazu GOLENHOFEN, HENSEL und HILDEBRANDT 1956).

Die Schwierigkeit einer brauchbaren direkten Registrierung der Kapillarströmung im Skeletmuskel beruht auf dessen geringer effektiver Parenchymdurchblutung. Diese beträgt weniger als 10 ml/100 g pro min.:

*Menschenmuskel:* 1.8—9.6 nach COOPER, EDHOLM und MOTTRAM (1955). *Hundemuskel:* 7.0—8.0 (Höchstwerte für den Ruhezustand) nach KRAMER (1941), 5.5 nach HATCHER und JENNINGS (1956 und 1957). *Katzenmuskel:* 2.5—5.0 nach LINDGREN (1957). Alle Werte in ml/100 g/min.

Bei einer effektiven Parenchymdurchblutung bis zu etwa 10 ml/100 g/min ist demnach der konvektive Wärmeabtransport durch die Kapillarströmung selbst zu gering, um  $\delta$  für eine zufriedenstellende Durchblutungsregistrierung ausreichend und genügend schnell zu vermindern. In Gewebe mit höherer Durchblutung wie z. B. der Leber (Durchblutung etwa 100 ml/100 g pro min, Werte s. bei GRAF, GOLENHOFEN und HENSEL 1957) oder dem Herzmuskel (Durchblutung 45—100 ml/100 g/min, KIESE und LANGE 1957) kann dagegen bei Anwendung des gleichen Verfahrens mit einer besseren Registrierung auch der Kapillarblutung gerechnet werden. Dabei darf für den Fall des Skeletmuskels jedoch nicht übersehen werden, dass die bei guten Sonderlagen hauptsächlich gemessene Arterien- und Venendurchblutung indirekt auch nur das Verhalten der Kapillardurchblutung wiedergibt, wenn man von den dort noch umstrittenen arterio-venösen Anastomosen absieht.

Die bei Ruhedurchblutung gemessenen  $\Delta\lambda$ -Werte führen zu einer Bewertung in gute und schlechte Sonderlagen je nachdem, ob  $\Delta\lambda_R$  kleiner oder grösser als  $1.0 \cdot 10^{-4}$  ist. Diese Einordnung ist unbedingt notwendig, weil sie die Grundlage dafür bildet, dass Messungen verschiedener Versuche verglichen werden können. Der Vergleich ist möglich, weil die Wärmeleitzahlen der un durchbluteten gesunden Skeletmuskulatur wie auch anderer gesunder parenchymatöser Organe (Leber, Niere, Milz, Herzmuskel) einen praktisch konstanten Wert von etwa  $11.0 \cdot 10^{-4}$  haben (GRAYSON 1952; LINZELL 1953; HENSEL, RUEF und GOLENHOFEN 1954; HENSEL und BOCK 1955; GRAF, GOLENHOFEN und HENSEL 1957; KIESE und LANGE 1957). Man kann deshalb sagen: Messungen, die von  $\Delta\lambda_R$ -Werten unter  $0.5 \cdot 10^{-4}$  ausgehen, sind zur Anzeige schneller Durchblutungsänderungen nicht geeignet, und

gleichbleibende Durchblutungswerte können durch die oft nicht völlig vermeidbaren geringen Schwankungen des Temperaturgradienten zwischen den beiden Lötstellen zu leicht verfälscht werden.  $\Delta\lambda_R$ -Werte von  $0.5-1.0 \cdot 10^{-4}$  stellen für manche Zwecke einen sicherlich bereits brauchbaren Ausgang dar. Erst ab  $\Delta\lambda_R = 1.0 \cdot 10^{-4}$  aber wird die Registrierung (ein genügend frequentes Registriersystem vorausgesetzt) das Durchblutungsverhalten ausreichend zuverlässig wiedergeben und dies bei weiter steigenden  $\Delta\lambda_R$ -Werten zunehmend besser tun. Ein exakter Vergleich der Messungen verschiedener Versuche ist nur möglich, wenn die Wärmeleitzahländerungen in allen Fällen von gleichgrossen  $\lambda$ -Werten (oder in Annäherung gleichgrossen  $\Delta\lambda_R$ -Werten) aus erfolgen. (So ist z. B. zu bezweifeln, ob Unterschiede im Kurvenverlauf bei schnelleren Durchblutungsänderungen nach Durchblutungsdrosselung eine allein biologische Ursache haben, wenn sie 2 Messungen entnommen werden, die in dem einen Fall von  $\Delta\lambda_R = 1.2 \cdot 10^{-4}$ , in dem anderen Fall aber nur von  $\Delta\lambda_R = 0.2 \cdot 10^{-4}$  ausgehen (s. GOLENHOFEN und HILDEBRANDT 1957 (1), Abb. 1). Eine weitere Zunahme von  $\Delta\lambda_R$  über  $1.0 \cdot 10^{-4}$  ist ohne wesentliche praktische Bedeutung, da von hier ab alle spontanen und reaktiven Änderungen des Stromzeitvolumens ausreichend und amplitudengetreu angezeigt werden. (Diese Zahlen gelten nur für Registrierungen, die mit der Metallsonde vorgenommen werden. Bei Verwendung von kunststoffumhüllten Wärmeleitmessern ist die Anzeigeverzögerung etwas grösser.)

Bei Versuchen an der Skelettmuskulatur des Menschen mussten wir die Sondenlage im Durchschnitt fünfmal verändern, ehe eine thermokonstante und genügend empfindliche Sondenlage mit  $\Delta\lambda_R$ -Werten von wenigstens  $1.0 \cdot 10^{-4}$  gefunden war (DIAMANT, GRAF und HOLMSTEDT 1957). Bei der Katze war das Auffinden einer brauchbaren Sondenlage noch erheblich schwieriger. Im Durchschnitt erhielten wir bei ihr eine solche erst nach 10—20-maligem Verschieben der Sonde. Dieser Unterschied erklärt sich einmal aus dem viel geringeren Volumen der Katzenextremität und dem dadurch bedingten grösseren axialen und radiären Temperaturgefälle in der Muskulatur (BRÜCK und HENSEL 1953). Zum andern mag auch die unphysiologische Lagerung der Tiere während der Durchblutungsmessung und die Narkose, in der die Körpertemperatur sehr leicht Schwankungen unterliegt (THAUER 1939; HENSEL 1955; GRAYSON und MENDEL 1956), von Bedeutung sein. Ob zusätzlich in der Narkose auch noch die Muskeldurch-

blutung vermindert wird, ist unseres Wissens bisher nicht untersucht worden. Am besten wird wohl das besonders schwierige Finden einer brauchbaren Sondenlage bei der Katze aus den anatomischen Verhältnissen verständlich. In ihrem Skeletmuskelparenchym verlaufen, wie uns die Präparationen zeigten, nur sehr wenige noch makroskopisch erkennbare Gefäße. Diese sind vielmehr fast nur in den Bindegewebssepten zwischen den einzelnen Muskeln zu finden. Ihr Volumen stellt nur einen kleinen Prozentteil des gesamten Muskelparenchymvolumens dar (nach PAPPENHEIMER (1955) soll das Volumen sämtlicher Muskelgefässe 4 % des Muskelvolumens betragen). Obwohl sich die effektive Parenchymdurchblutung der menschlichen von der der Katzenmuskulatur praktisch nicht unterscheidet (s. Seite 66), ist die absolute Blutmenge und damit auch das Volumen der hauptsächlichen Gefäße verschieden. Die Wärmeleitsonde wird daher in der menschlichen Muskulatur eher als in derjenigen der Katze auf Gefäße von makroskopisch sichtbarer Grösse treffen. Die besten Sondenlagen werden diejenigen sein, bei denen die Sondenspitze nicht tief im Muskelparenchym, sondern in einem intermuskulärem, grosse Gefäße enthaltenden Bindegewebsseptum liegt.

Dieser Befund berechtigt zu der Frage, ob eine in die Skeletmuskulatur eingeführte Wärmeleitsonde bei genügend grossem  $\Delta\lambda_R$  tatsächlich nur die Skeletmuskeldurchblutung registriert, da möglicherweise die grösseren Arterien und Venen auch Anteil an der Hautdurchblutung haben könnten. Unsere Versuche können diese Frage nicht entscheiden.

Mit der Wärmeleitmessung wird jeweils nur die Durchblutung in einem kleinen Teil des gesamten Organs registriert. Die Wärmeleitzahländerungen müssen daher keineswegs den Änderungen der Gesamtdurchblutung entsprechen. Sie sind bei  $\Delta\lambda_R > 1.0 \cdot 10^{-4}$  diesen aber oft sehr ähnlich und weichen auch in den Extremfällen nur um 50 % von den Gesamtdurchblutungsänderungen ab (Abb. 3). Das wird dadurch verständlich, dass bei guten Sondenlagen vorwiegend die Durchblutung von Arterien und Venen, die eine grössere Parenchymregion versorgen, registriert wird, und für den Skeletmuskel ein auch in allen grösseren Abschnitten annähernd gleichartiges Durchblutungsverhalten angenommen werden kann (gleichsinniger Kurvenverlauf bei fortlaufender Muskeldurchblutungsregistrierung in einer Wade des Menschen mit 2 Wärmeleitsonden (GOLENHOFEN, HILDEBRANDT und SCHERER 1956; GOLENHOFEN und HILDEBRANDT 1957 (2)).

Es erscheint daher berechtigt, die Ergebnisse von Wärmeleitzahlmessungen in der Skelettmuskulatur, die von einem  $\Delta\lambda_R$  von mindestens  $1.0 \cdot 10^{-4}$  ausgehen, dem Verhalten der Gesamtdurchblutung der Muskulatur annäherungsweise gleichzusetzen.

Eine bessere quantitative Übereinstimmung mit dem Verhalten der Gesamtdurchblutung wäre zu erwarten, wenn der durch den Wärmeleitmesser aufgeheizte Gewebsbezirk grösser als bei der HENSEL'schen Wärmeleitsonde ist. So konnten beispielsweise HENSEL und BENDER (1956) bei Verwendung eines Wärmeleitmessers für die Haut, der einen Gewebsbereich von 1—3 cm<sup>2</sup> für die Messung erfasst, die  $\Delta\lambda$ -Werte direkt in ml Hautdurchblutung/100 ml Haut/min eichen. Im Gewebsinneren würde eine modifizierte Gewebsheizung mittels hochfrequentem Wechselstrom (GOLENHOFEN 1956), die — im Gegensatz zur punktförmigen Heizung bei der von uns verwandten Wärmeleitsonde — einen grösseren Gewebsbezirk erwärmt, zu besserer quantitativer Auswertbarkeit führen können.

Die im Vergleich zu Messungen mit einer metallumhüllten Wärmeleitsonde tragere Durchblutungsanzeige mit einer in Kunststoff eingefassten Wärmeleitsonde wird mit der Differenz der Wärmeleitfähigkeit der Materialien erklärt. Deren Werte sind:

V2A-Stahl (Chrom-Nickel-Stahl 18/8)  $\lambda = 350 \cdot 10^{-4}$  cal · cm<sup>-1</sup> · sec<sup>-1</sup> · °C<sup>-1</sup>.  
Palavit  $\lambda = 5.6 - 8.0 \cdot 10^{-4}$  cal · cm<sup>-1</sup> · sec<sup>-1</sup> · °C<sup>-1</sup> (nach FRITZ 1957).

Die Wärmemenge, die die Sondenoberfläche an das kältere Gewebe abgibt, muss ihr aus dem Inneren der Sonde durch Leitung zuströmen. Sie wird in der Zeiteinheit umso geringer sein, je kleiner die Wärmeleitzahl des Sondenmediums ist, was LINZELL (1953) in Durchströmungsversuchen mit kupfer- sowie polyäthylenbedeckten Wärmeleitmessern auch experimentell belegen konnte (Kupfer (Handelskupfer)  $\lambda = 8,890 \cdot 10^{-4}$  cal · cm<sup>-1</sup> · sec<sup>-1</sup> · °C<sup>-1</sup>, Polyäthylen  $\lambda = 8.0 - 11.0 \cdot 10^{-4}$  cal · cm<sup>-1</sup> · sec<sup>-1</sup> · °C<sup>-1</sup> (nach FRITZ 1957).

Für die Auswertung von Versuchen mit flexiblen, in Palavit und Polyäthylen eingefassten und zur Anwendung in der Leber bestimmten Wärmeleitsonden (GRAF, GOLENHOFEN und HENSEL 1957) sind diese Ergebnisse wichtig. Obwohl der schlechte Wärmeübergang in solchen Sonden wegen der höheren Wärmeleitzahl in der durchbluteten Leber an Bedeutung verliert, dürfen die Amplituden der Durchblutungsänderungen auch hier nur als Mindestwerte angesehen werden. Denn neben dieser, auf die Materialeigenschaften des Messgerätes zurückzuführenden Anzeigeverminderung wird der Wert auch noch durch die Abweichung von der angenommenen linearen Beziehung zwischen  $\Delta\lambda$  und Stromzeitvolumen vermindert, die der prozentualen Eichung der Durchblutungsänderungen zugrundegelegt ist (HENSEL, RUEF und GOLENHOFEN 1954; GRAF, GOLENHOFEN und HENSEL 1957). Bei

relativ geringem Stromzeitvolumen wie in der Skelettmuskulatur sind Palavit- und Polyaethylensonden unbrauchbar. Hier können nur mit metallumhüllten Wärmeleitmessern ausreichende Messergebnisse erzielt werden.

### Zusammenfassung.

1. In der Hinterextremitätenmuskulatur narkotisierter Katzen wurden Wärmeleitzahl (mit der *Wärmeleitsonde* nach HENSEL) und Stromzeitvolumen synchron und fortlaufend registriert.

2. Die Wärmeleitsonde zeigt schnellere Durchblutungsänderungen (pharmakologisch bedingte und spontane) in der Skelettmuskulatur nur dann ausreichend amplitudentreu an, wenn die Wärmeleitzahlerhöhung  $\Delta\lambda$  bei Ruhedurchblutung ( $\Delta\lambda_R$ ) mindestens  $1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  beträgt. Von kleineren  $\Delta\lambda_R$ -Werten ausgehend werden schnellere Durchblutungsänderungen zunehmend trüger und daher mit zu kleiner Amplitude registriert.

3. Ein genauer Vergleich der Messungen verschiedener Versuche ist nur möglich, wenn die Wärmeleitzahländerungen von gleichgrossen  $\lambda$ -Werten (oder in Annäherung gleichgrossen  $\Delta\lambda_R$ -Werten) aus erfolgen.

4.  $\Delta\lambda_R$  ist von der Entfernung makroskopisch erkennbarer Gefäße zur Sondenspitze abhängig. Makroskopisch erkennbare Gefäße liegen bei  $\Delta\lambda_R > 1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  unmittelbar an der Sondenspitze. In der Skelettmuskulatur kann mit der Wärmeleitsonde direkt nur die Durchblutung von makroskopisch sichtbaren Gefäßen, also von Arterien und Venen, in zufriedenstellendem Masse angezeigt werden, nicht dagegen die Kapillarströmung.

5. Makroskopisch erkennbare Blutungen als Folge des Einstichs der Wärmeleitsonde wurden bei etwa 300 Sondeneinstichen nur dreimal beobachtet.

6. Die relativen (prozentualen)  $\Delta\lambda$ -Änderungen sind den relativen (prozentualen) Änderungen der Gesamtdurchblutung ähnlich, wenn die Wärmeleitmessung von  $\Delta\lambda_R$ -Werten  $> 1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot {}^\circ\text{C}^{-1}$  ausgeht.

### Summary.

1. Thermal conductivity (with the use of HENSEL's „Wärmeleitsonde“) and total blood flow (ml/min) in the hind leg muscles were recorded simultaneously and continuously in anesthetized cats.

2. Rapid changes (pharmacologically induced or spontaneous) in total muscle blood flow were accompanied by swift changes in thermal conductivity only in those cases in which the thermal conductivity increment  $\Delta\lambda$  at resting blood flow ( $\Delta\lambda_R$ ) was at least  $1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot ^\circ\text{C}^{-1}$ . At lower  $\Delta\lambda_R$  values the changes in thermal conductivity became more and more delayed.

3. Results from different experiments can be exactly compared only in cases with equal  $\lambda$  values (or approximately equal  $\Delta\lambda_R$  values).

4.  $\Delta\lambda_R$  depends on the volume of macroscopically visible blood vessels and the distance between those vessels and the tip of the „Wärmeleitsonde”. At  $\Delta\lambda_R$  values of  $1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot ^\circ\text{C}^{-1}$  and higher, macroscopically visible vessels were found in direct contact with the tip of the needle. The results suggests that in skeletal muscle the „Wärmeleitsonde” records adequately the blood flow in large vessels (arteries and veins) rather than the capillary blood flow.

5. Gross hemorrhages in muscle tissue were found only in 3 cases in a total of 30 experiments with about 300 introductions of the needle.

6. The relative (percentual) changes in  $\Delta\lambda$  closely accord with the relative (percentual) changes in total muscle blood flow provided that recording of thermal conductivity starts from  $\Delta\lambda_R$  values of at least  $1.0 \cdot 10^{-4} \text{ cal} \cdot \text{cm}^{-1} \cdot \text{sec}^{-1} \cdot ^\circ\text{C}^{-1}$ .

Die Untersuchungen erfolgten mit Unterstützung des SSP:s fond för medicinsk forskning, für die an dieser Stelle gedankt sei.

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## The Physical Work Capacity of Workers 50—64 Years Old.

By

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In connection with medical and hygienic improvements, the average life span within the last fifty years has increased considerably, which means a shift in the average age of the population. The physical work capacity decreases successively with increasing age, and consequently more and more individuals with a reduced physical work capacity can be found on the labour market. In enterprises where middle aged and older workers are employed, and where in many jobs a considerable demand on the physical work capacity can be expected, it becomes more and more evident that the older workers should be placed in the lighter jobs in order to avoid overstraining. These problems are discussed, among others, by COWDRY (1952), HENRY (1952), CHRISTENSEN (1955), KAHLSON (1956) and ÅSTRAND (1956).

The most important and detailed investigation with regard to the physical work capacity of higher age groups was done by ROBINSON (1938). In his study, which included 18 subjects over 50 years of age, 7 were 51.0, 8 were 63.1, and 3 were 75.0 years. These people came under the heading of "business and professional men", and consequently in their daily work there was no heavy demand for a great physical capacity, defined as that capacity required to perform a certain physical work for a long time. Since such a work is performed primarily by energy released from oxidative processes, the physical work capacity is usually measured

as the capacity of maximal oxygen intake. For the 51.0 years old group the physical work capacity defined in this way was 75 % of the value for 11 persons with an average age of 24.5 years, for the 63.1 years old group it was 67 % and for the 75.0 years old group, 48 %.

In the younger age group, the physical work capacity varies considerably with different degrees of training of the muscles, and the respiratory and circulatory organs. Since the desire for spontaneous physical activity in the average person decreases with age, it is probable that the decline in the physical work capacity can be attributed partly to decreased spontaneous activity. For these reasons it is possible that this "ageing process" could partly be checked if people would maintain a certain amount of physical training. Consequently one would expect to find in workers employed in professions which require physical activity a greater average physical work capacity than ROBINSON found. Another reason for such a finding could be that a selection had been made so that the workers who were physically weaker had been sorted out at an earlier state.

On the other hand, the heavier jobs might be too heavy and have the effect of wearing out the individual at a relatively young age. After engagement for 30—40 years in such work one may then expect to find a lower physical work capacity and also a greater incidence of degenerative diseases, for instance, heart disease.

In the study presented here a group of workers over 50 years of age were investigated with respect to physical work capacity. Clinical examination at rest and during work of heart and lung functions were also included in the investigation. The results of these later examinations, however, will be dealt with in another publication (ÅSTRAND, 1958).

### Materials.

As subjects 81 men were used of which 46 were 50—54 years old, 27 were 55—59 and 8 were 60—64 years old. All of them were truck drivers for breweries in Stockholm, and had, on the average, been in the same type of job for 21.2 years. Moreover before they started as truck drivers all of them had been employed in relatively heavy jobs for the greater part of their lives.

In all there were about 100 truck drivers over 50 years of age

Table 1.

*Body height, body weight and lung volumes in the different age groups of subjects.*

Age	Number	Body height cm	Body weight kg	Vital cap. litres	Residual volume litres
50—54 years ....	46	175 $\pm$ 1 6.0	76.4 $\pm$ 1.6 10.9	4.72 $\pm$ 0.11 0.70	1.89 $\pm$ 0.05 0.35
55—59 years ....	27	175 $\pm$ 1 6.3	78.3 $\pm$ 1.8 9.1	4.59 $\pm$ 0.11 0.53	2.09 $\pm$ 0.09 0.42
60—64 years ....	8	174 $\pm$ 1 3.8	68.8 $\pm$ 3.5 10.6	4.17 $\pm$ 0.20 0.48	2.35 $\pm$ 0.25 0.61

The figures denote the mean value  $\pm$  the standard deviation of the mean and the standard deviation.

who were employed by these breweries. Those who were not investigated were for some reason not able to take part.

These truck drivers distributed daily approximately 50—80 cases of beer each weighing about 43 kg or 100—125 cases each weighing about 19 kg. The work consisted of transporting the beer by truck to the consumer, and usually the cases were carried on the back of the driver from the truck to the unloading place. Furthermore, the work of the driver included loading and unloading the truck at the brewery. The carrying of the cases on the back demands, as a steady state work, an oxygen consumption of about 1.5 litres/min. (level loading place) to 2.5 litres/min. (carrying upstairs), and is therefore rather heavy work although the periods of activity are of relatively short duration. Actually a steady state is never reached (see LUNDGREN, 1957).

One consequence of the type of job is that the material here described cannot be representative for the whole population in this age group. It must be considered as probable that physically weak subjects leave this work before reaching the age of 50 years. Also the milieu in the work is different from that of other workers in the respect that beer is easily accessible. This can be suspected to lead to a higher consumption of alcohol than normal. However, the essential thing is that the group is normal from a social point of view, and occupied in a relatively heavy work.

Some anthropologic data of the different age groups of subjects and their lung volumes are summarized in Table 1.

### Methods.

A careful clinical examination preceded the physiological investigation in the laboratory. The work test of CHRISTENSEN and FORBES (1937) with increasing work loads on a bicycle ergometer was used with the slight modification that the time for the work at each load was limited to about six instead of eight minutes in the original test. The rest period between the work loads was about four minutes.

The cycling was performed on a KROGH bicycle ergometer with continuous registration of the pedal frequency. This enables an exact determination of the work to be made also for subjects having difficulty to follow the metronome pace.

Each subject was tested on two different occasions. The objective was for each subject to work two times with a work load of 600 kpm/min., one or two times with 900 kpm/min., and if possible, one or more times with a higher work load. During the last experiment the subjects attempted to reach their maximal levels.

After a "steady state" was reached with regard to the heart rate, the *oxygen intake* was determined at each work load by means of the DOUGLAS bag method. A more detailed discussion of physical work capacity, the methods used and the error of the methods are given by ÅSTRAND (1952).

*Heart rate* was determined each minute with the help of a stethoscope; a "steady state" was usually reached after about 5 min. The heart rate used for each respective work load was usually a mean value calculated from the heart rate determined with a stethoscope and the heart rate from ECG recordings before and during the oxygen intake determination. If these values varied by more than 4 beats the highest value was used.

Approximately one and two minutes after the end of each work load, a blood sample was taken from a warmed fingertip for determination of the *lactic acid concentration* (according to STRÖM's modification (1949) of BARKER and SUMMERSON's colorimetric method). The error of the method for a single determination calculated from 30 duplicate determinations was, for concentrations between 0—25 mg %,  $\pm 1.26$  (7.9 %), 25—50 mg %,  $\pm 1.66$  (4.5 %) and over 50 mg %,  $\pm 1.92$  mg % (3.1 %).

Usually the values for the heart rate, oxygen intake, pulmonary

ventilation and blood lactate concentration for a given work load were lower on the second experimental day than on the first; mechanical efficiency was accordingly higher. The average mechanical efficiency was 22–23 per cent the second day, which means it was about the same as for younger persons (ÅSTRAND, 1952). In many cases the difference in oxygen intake and in heart rate from day one to day two was 0.2 litres/min. and 15 or more beats/min. respectively. The probable explanation is that the older subjects were more tense during the first experiment; such a reaction is more unusual with younger subjects. The average difference was not statistically significant. However, this observation is important and shows that at least 2 tests ought to be made before any definite conclusions are drawn with respect to the physical work capacity of older people. In the calculations the values from the second experimental day were used. When only one determination was made at a given work load, and this determination was made on the first day, the values in some cases were apparently abnormal. In such cases these values were not used in the calculations of the mean values.

### Results and Discussion.

The tabulated individual results can be obtained from this department upon application.

#### A. Maximal work load.

As was expected the main difficulty was to get the subject to work as close to his maximal capacity as possible. One reason was that it was impossible to predict from the submaximal values of heart rate the highest work load the subject would be able to maintain for 5–6 min.; this in marked contrast to the results obtained on young individuals. As an objective control to make sure that the work had been close to maximal, the lactic acid concentration in the blood at the end of work was used.

The average *maximal heart rate* measured in 45 subjects 50–54 years old was 161 beats/min. The corresponding value for 22 subjects 55–59 years old was 158 and for 6 subjects 60–64 years old, 158 beats/min. The average *maximal oxygen intake* for the three age groups was 2.55, 2.43 and 2.14 l/min. and the *maximal*

Table 2.  
Oxygen intake, heart rate and lactate concentration in the blood during maximal and submaximal work. ÅSTRAND's and ROBINSON's values are included.

	Maximal work				Submaximal work							
	Work load kpm/min.	Oxygen intake l/min. STPD	Oxygen intake min/kg/min. STPD	Heart rate per min.	Lactic acid conc. mg %	615 kpm/min.	Oxygen intake l/min. STPD	Heart rate per min.	Lactic acid conc. mg %	917 kpm/min.	Oxygen intake l/min. STPD	Heart rate per min.
ÅSTRAND 1952 20-33 years	n = 42 1,800	4.11 ± 0.06 0.37	58.6 ± 0.7 4.1	n = 42 19.4 ± 2 10	n = 42 112 ± 3 20	n = 45 1.02 ± 0.02 0.12	n = 42 119 ± 2 15	n = 46 23 ± 1 8	n = 46 2.22 ± 0.02 0.11	n = 41 146 ± 2 13	n = 44 3.9 ± 2 10	n = 44 39 ± 2 10
1. ÅSTRAND 1957 50-54 years	n = 43 1,122 ± 20.8	2.55 ± 0.05 0.32	33.9 ± 0.8 5.2	n = 44 16.1 ± 2 10	n = 45 58 ± 2 10	n = 42 1.61 ± 0.02 0.10	n = 42 116 ± 3 16	n = 46 23 ± 2 10	n = 46 2.18 ± 0.03 0.12	n = 22 144 ± 3 16	n = 23 42 ± 3 15	n = 24 39 ± 2 15
55-59 years	n = 24 1,060 ± 37	2.43 ± 0.07 0.32	30.9 ± 0.9 4.3	n = 23 15.8 ± 2 10	n = 22 55 ± 2 10	n = 24 1.61 ± 0.02 0.10	n = 27 116 ± 3 16	n = 27 23 ± 2 10	n = 27 2.18 ± 0.03 0.12	n = 23 144 ± 3 16	n = 23 42 ± 3 15	n = 24 39 ± 2 15
60-64 years	n = 6 942 ± 55 134	2.14 ± 0.14 0.31	31.9 ± 2.0 4.4	n = 5 15.8 ± 4 10	n = 6 60 ± 5 11	n = 5 1.52 ± 0.03 0.07	n = 6 118 ± 9 21	n = 6 26 ± 4 10	n = 6 2.15 ± 0.04 0.09	n = 4 149 ± 5 11	n = 5 54 ± 6 13	n = 5 54 ± 6 13
All subjects 50-64 years	n = 73 1,087 ± 19	2.48 ± 0.04 0.34	32.8 ± 0.6 5.0	n = 72 16.0 ± 1 10	n = 75 57 ± 2 13	n = 65 1.61 ± 0.01 0.11	n = 79 118 ± 2 15	n = 79 23 ± 1 9	n = 67 2.20 ± 0.01 0.11	n = 72 145 ± 2 14	n = 73 41 ± 2 13	n = 73 41 ± 2 13
ROBINSON "51" years . .	n = 7 2.63	n = 7 38.4	n = 7 n = 8 2.35	n = 7 173	n = 7 n = 8 34.5	n = 7 n = 8 165	n = 7 n = 8 58	n = 7 n = 8 58	n = 7 n = 8 58	n = 7 n = 8 58	n = 7 n = 8 58	
"63.1" years . .												

<sup>1</sup> Åstrand 1952 used 900 kpm/min.  
The figures denote the mean value ± the standard deviation of the mean value and the standard deviation.  
n = number of subjects.

Max. oxygen intake l/min STPD

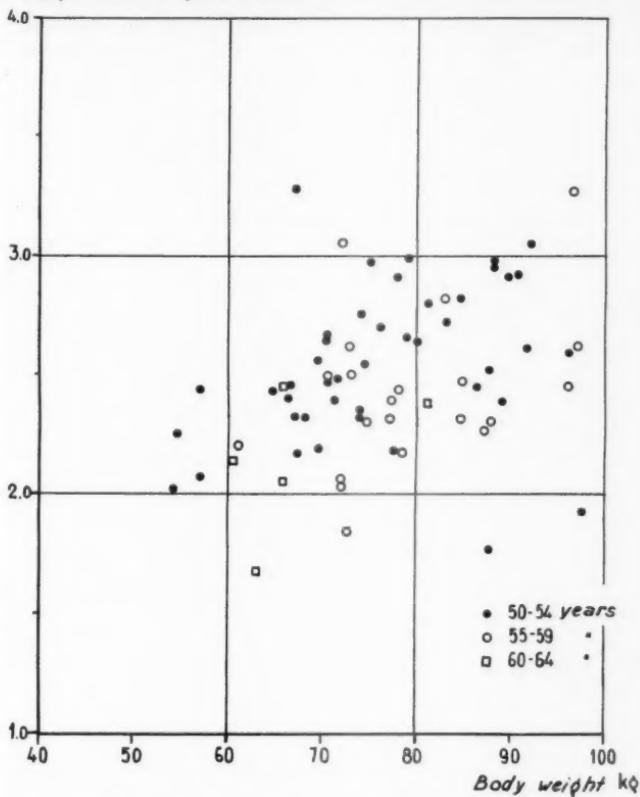


Fig. 1. The maximal oxygen intake in relation to body weight.

lactic acid concentration 58, 55 and 60 mg %, respectively, see Table 2. For comparison both ROBINSON's and ÅSTRAND's values are included.

The average maximal oxygen intake for the first group of subjects was only 62 % of ÅSTRAND's values for younger subjects; for the second group it was 59 % and for the third group 52 %. The maximal values for heart rate and lactic acid concentration were also relatively low compared to the maximal values of the younger subjects. These results are discussed later.

Those low values raise the question whether the work was

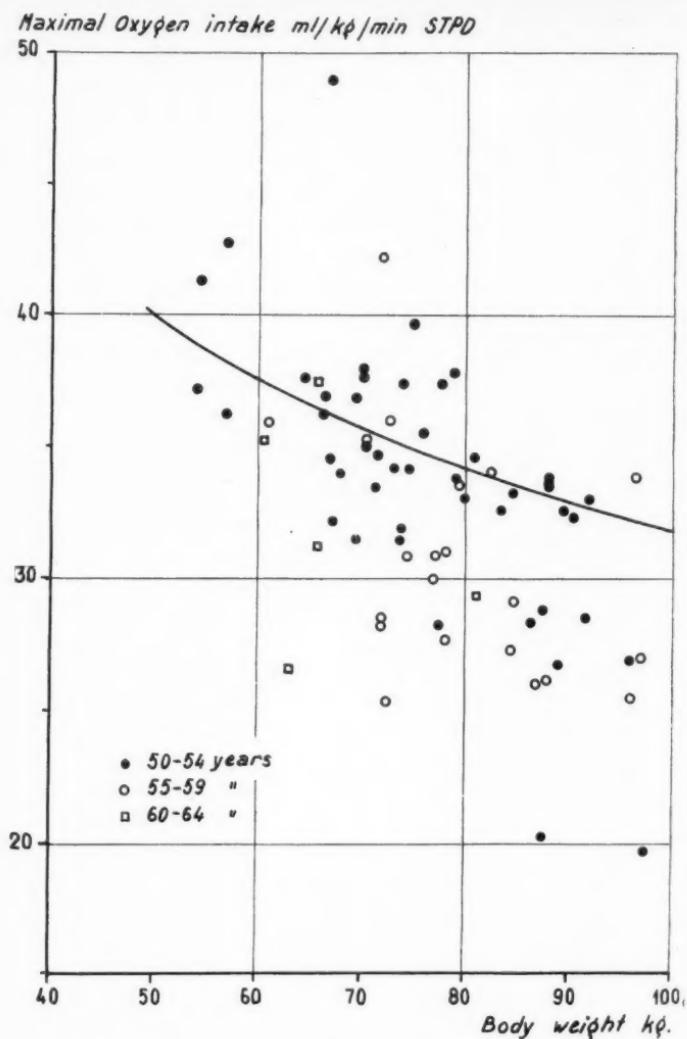


Fig. 2. The maximal oxygen intake per kg body weight in relation to body weight. The drawn line shows the relationship if aerobic capacity was a function of body mass raised to the power of 2/3.

maximal or not. By analysing ÅSTRAND's results it was found that younger subjects on the average made use of 90 % of their maximal oxygen intake when they had a lactic acid concentration in the blood of 50 mg %, that is, they really worked close to maximal. If the same relationship is true for the older group, they should have made use of about 90 % of their aerobic capacity at a lactic acid concentration of 57 mg %. This means that the older group should be able to increase their oxygen intake by no more than about 10 %, for example from 2.55 to 2.80 l/min. for the first group. This possible change in  $O_2$  intake would not affect the conclusions. Owing to the uncertainty in this difference the directly measured values were used in all tables, figures and in the discussions.

The physical work capacity (maximal oxygen intake) is on the whole increased with increasing body size for young, healthy persons with normal proportions between body weight and height. Such a person with a body weight of 50 kg has a lower maximal capacity for oxygen intake than one with 75 kg. This relationship is very important, especially if the labour involves moving of weights, as for instance when carrying loads.

The maximal oxygen intake in relation to body weight for the older subjects is shown in Fig. 1. This shows the tendency for a higher oxygen intake with greater body weight. According to VON DÖBELN (1956 b), the maximal oxygen intake for young healthy people is proportional to the lean body mass raised to the power 2/3. From Fig. 2 it is obvious that the oxygen intake per kg body weight decreases with increasing body weight. The values are grouped around a curve with a steeper slope than one would expect if oxygen intake was proportional to body weight raised to 2/3 (see the drawn line). The conclusion is that the body weights of the subjects were not a good measure of their lean body mass.

In Fig. 3 the maximal oxygen intake is plotted against body height. The drawn line indicates the slope of the curve to be expected if maximal metabolic rate would be proportional to the length scale squared. By comparison of Fig. 1 and 3 it is seen that height and especially height squared is a better anthropologic reference figure for maximal metabolic rate in older individuals than total body weight. The maximal oxygen intake per unit body height squared in the present material is  $0.81 \pm 0.012$  l/m<sup>2</sup>, S. D. 0.098.

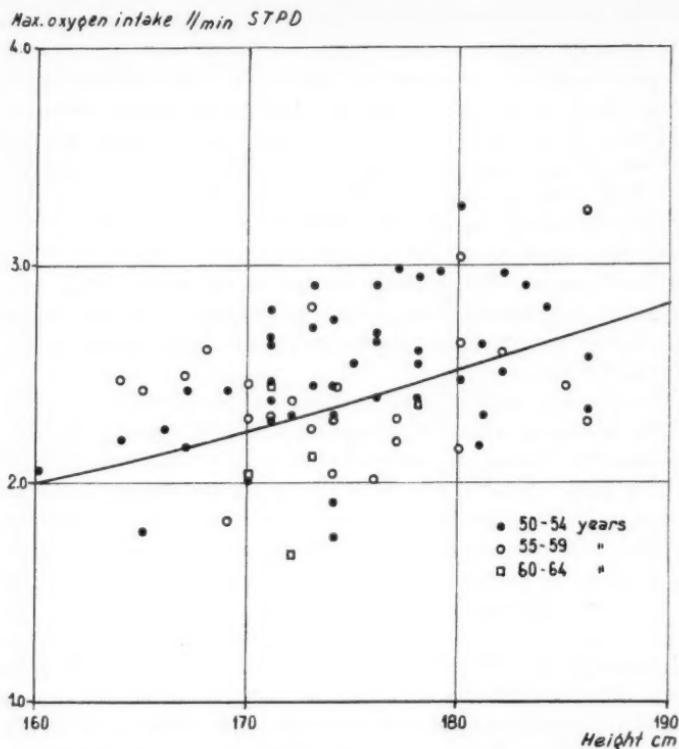


Fig. 3. The maximal oxygen intake in relation to body height. The drawn line shows the relationship if aerobic capacity was a function of height squared.

In ÅSTRAND's material from 1952 the coefficient of variation for the maximal oxygen intake is  $0.37/4.11 = 9\%$  and for the maximal oxygen intake per kg body weight  $4.1/58.6 = 7\%$ . In the present material the corresponding figures are  $0.34/2.48 = 13.7\%$  and  $5.0/32.8 = 15.2\%$ . The figure for the maximal oxygen intake per unit body height squared is  $0.098/0.81 = 12.1\%$ . Neither body weight nor height is accordingly an ideal parameter for the maximal oxygen intake of older people. A calculation of the maximal oxygen intake per kg body weight is, however, still preferable for these people as they in their job have to carry their own body weight.

When ROBINSON's older subjects were compared with his 20-30

year old group, there was found a maximal oxygen intake of 75 % (per kg body weight 79 %) for subjects 51.0 years old, and 67 % (71 %) for subjects 63.1 years old. His older subjects were lighter than his younger subjects. ROBINSON's values for the oxygen intake per kg body weight are about 12 % higher than in the present material, but the absolute values for the oxygen intake agree very well, as can be seen in Table 2 (see also the lactic acid values). If in the present investigation 38 subjects with over weight are excluded from this comparison the difference is reduced to 6 %.

The present group with heavy labour has a work capacity of about the same size as ROBINSON's group of "business and professional men". If one presupposes that ROBINSON's subjects are representative of lighter labour from a physical viewpoint, the conclusion can be drawn that comparatively heavy work did not change the physical work capacity. The problem in both investigations was to decide whether or not the values were maximal. The difference in aerobic capacity between the younger groups of ROBINSON and ÅSTRAND is discussed by ÅSTRAND (1952).

#### B. Submaximal work load.

In the present investigation the heart rate for the 50—54 year old subjects was, at an average work load of 615 kpm/min., 119 beats/min., and the oxygen intake was 1.62 l/min. at a lactate concentration of 23 mg %. The corresponding values for the 55—59 and 60—64 year old subjects are given in Table 1, as are the values at the average work load of 917 kpm/min.

With children and younger adults it was shown (ÅSTRAND, 1952) that the pulse frequency in a steady state increased almost rectilinearly with increasing work load. This was valid for adults for an increase from about 125 to about 170 beats/min. With the use of these and other results from this laboratory a nomogram was constructed (ÅSTRAND and RYHMING, 1954). With the help of this nomogram one can predict, from the pulse frequency in a steady state at a *submaximal* work load, the *maximal* oxygen intake of healthy 18—30 year old people with a standard deviation less than  $\pm 10\%$ .

If the nomogram is applied to the subjects in this investigation it appears, that the predicted figures for maximal oxygen intake are much higher than those experimentally found. The pulse rate

of 145 at an oxygen intake of 2.2 l/min. which is found in the subjects of 50—59 years of age, would correspond to a maximal oxygen intake of 3.5 l/min. This figure is close to 1 litre more than what is actually found. The nomogram of ÅSTRAND and RYHMING is based on the assumption that the maximal pulse rate is about 195 beats per minute. In this study the pulse rate at maximal work is close to 160.

These facts raise the following question. Is the reaction of the heart rate to submaximal and maximal work altered with ageing or is the effect of ageing on physical work capacity mainly due to a lowering of the maximal possible heart rate? The pulse reaction to a load of about 900 kpm/min. in this material is definitely higher than what ÅSTRAND found 1952 on young, specially well trained subjects. In an investigation of Swedish air pilots, however, v. DÖBELN, ENGSTRÖM and STRÖM (1958) found a pulse reaction of 145 to 900 kpm/min. of pilots of all ages from 20—50 years. The pulse reaction to submaximal work in that material was independent of age. Consequently the value found in our older subjects is normal, even if different from ÅSTRAND's and not necessarily a symptom of ageing.

Regarding the possibility that the pulse response to work could be changed by ageing, it is of interest to compare the older with the younger with respect to the increase of the heart rate in the early phases of work. As a matter of fact in comparing the rate of change in heart rate in a group of older subjects with that of a group of well trained, younger athletes, in going from rest level to a steady state level of 1) 115—125 and 2) 140—150 no significant differences were observed (see Fig. 4).

If the pulse reaction of the older subjects to submaximal work were different from that one in the younger subjects on which the nomogram is based, the maximal  $O_2$  intake predicted from the pulse reaction to a lower load would be different from that one predicted from a higher load. This is not the case. Whether estimated from the pulse reaction to a work load of about 600 kpm/min. or 900 kpm/min. the predicted figure is the same close to 3.5 l/min., which figure is much too high. This means, that the maximal oxygen intake is reached at a lower level with a corresponding lower heart rate in the older than in the younger subjects with the same pulse reaction to submaximal work.

A possible explanation for this is that the maximal pulse rate and consequently the maximal minute volume may be influenced

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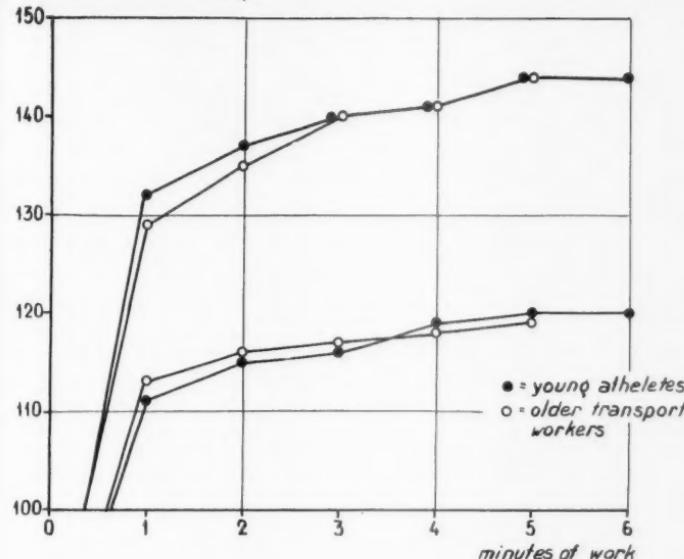


Fig. 4. The increasing heart rate in the early phases of work in a group of older subjects and in a group of well-trained, young athletes.

by the "ageing process" (compare ROBINSON). v. DÖBELN (1956 a) has claimed, that there exists a relationship between the maximal linear velocity of the blood in the aortic ostium and coronary blood flow. If the maximal coronary blood flow, expressed as percentage of the total blood flow, is lower in the older subjects this would mean, that the maximal linear velocity of the blood would be lowered. If the stroke volume is the same the consequence would be an increase in time for the systole and consequently a lowering of the heart rate and the minute volume. The findings in this study do not contradict this hypothesis.

Whether this hypothesis is true or not the fact remains that the nomogram of ÅSTRAND and RYHMING cannot be used in prediction of maximal oxygen intake for older people. At least this holds true for subjects who can not reach a heart rate of 195/min. during work. For the same reason it is incorrect to estimate the work capacity of older people by extrapolating from a chosen work load to a work load which they should be able to perform at an arbitrarily chosen heart rate, e. g. 170 beats/min., and then

directly compare such results to those obtained on younger subjects.

### Summary.

Eighty-one of one hundred 50—64 years old transport workers for breweries in a district of Stockholm were examined with respect to physical work capacity. The subjects were tested on a bicycle ergometer at increasing work loads, the heaviest of which caused the subject to be exhausted after about 6 minutes work. The following conclusions could be drawn from the data obtained.

1) The pulse reaction to a submaximal work load did not differ from that one of younger subjects. Mean heart rate at about 900 kpm/min. was about 145 beats/min.

2) Maximal work was reached at a lower work load than in younger subjects with the same pulse reaction to submaximal work.

3) The heart rate at maximal work was about 160 beats per minute at an oxygen intake of about 2.5 litres per minute.

4) The physical work capacity of individuals of different age can not be estimated and compared only on the basis of the pulse reaction to submaximal work.

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